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ON THE ORIGIN OF SNAKES BASED ON GEOMETRIC MORPHOMETRICS: MORPHOLOGY, PALEONTOLOGY, PHYLOGENY, ECOLOGY, AND DEVELOPMENT.

Filipe Oliveira da Silva

DOCTORAL DISSERTATION

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ABSTRACT

The title of this dissertation makes a direct reference to the most influential book in the history of biology published with the title "On The Origin of Species by Means of Natural Selection" (Darwin, 1859). Snakes are intriguing organisms, feared and loved, yet their ecological origin and evolutionary radiation remained uncertain for centuries. This debate has since the late 1990s received renewed attention due to controversies about the interpretations of fossils, the homology of anatomical structures, paleoecologies, and unstable phylogenetic topologies.

The publications included in this dissertation were the first to use geometric morphometrics of squamate skulls to address the ecological origin and radiation of snakes from lizards. I sampled, quantified, and compared numerous taxa, lineages, and developmental stages of lizards, snakes, and tuatara.

I evaluated four hypotheses. *i*: skull shape convergence evolved between lizards and snakes among their fossorial species; *ii:* skull shapes and ecologies are correlated so that paleoecologies could be estimated from skull shape parameters; *iii:* snakes evolved from either a fossorial, marine, or terrestrial ancestor; and *iv:* heterochrony underlies the snake skull shape development, consequently, evolution.

In agreement with set expectations based on qualitative data, fossorial lizards and snakes were highly convergent. Moreover, a significant correlation was detected between skull shapes in general and the range of coded habitats. Thus, it was possible to estimate with high statistical confidence the ancestral ecology of snakes from skull shape parameters. Surprisingly, snakes were estimated to have most likely evolved from a terrestrial lizard-like ancestor. The crown-snake ancestor was most likely a small and fossorial (not as specialized as observed in extant blind snakes). A marine origin was rejected on the grounds of our data and results. Importantly, the new ecological scenario has broken the traditional thinking about the origin of snakes because the early ancestral ecologies were significantly different. Before, stem and crown ancestors were commonly equated in their ecology. Finally, heterochrony was detected. It is most likely associated with the process of peramorphosis and linked with a mechanism of acceleration in skull shape development in alethinophidian snakes. Conversely, scolecophidians were either paedomorphic or have simply retained the ancestral condition for the rate of development that is seen among lizards.

From those results, skull form and function are seemly correlated, but the same ecologies seen in lizards are related to different skull and body shapes in snakes, except for most fossorial taxa. Thus, it was hypothesized here that snakes broke the developmental constraints that kept other fossorial squamate forms limited within the lizard morphospace. A fossorial ancestry for snakes did not fully limit their posterior increase in disparity, ecological radiation, and skull innovations. Besides, the radiation of snakes seemly reflects a balance between the acceleration of the skull development and the influence of natural selection that is associated with habitat, but,

most importantly in later snake evolution, dietary shifts. Integrated skull shape changes, but particularly those observed on the quadrate bone (anteroposterior elongation of the shaft and projection of its ventral articular surface), are compatible with the evolution of large-gape sizes that would have contributed to caenophidian snakes, boas, and pythons to prey on large homeothermic animals (mammals and birds). This scenario is also supported by the estimation of the ancestral skulls - the deep and crown snake ancestors both most likely had a limited gape-size. Skull shape changes can be then seen as a precondition for the successful radiations of alethinophidian snakes. Ultimately, the ophidian skull can be considered an evolutionary novelty.

Additionally, in-depth inspections of the literature as further detailed in this dissertation, revealed core evolutionary misconceptions that seem to hamper ecological interpretations and have provided misleading ecological hypotheses. They include misinterpretations of phylogenies and the type of ecological information that can be extracted from fossils both concerning the ecology and phenotype of snake ancestors. Those misconceptions are linked with the "sister-group fallacy"; reductionist views on the mode of phenotypic evolution as linear (basal/derived, primitive/advanced) as well as purely gradual (only small additive changes in a gradient); and fossils as "missing links" of a sequence of phenotypic changes, even if implicitly, including a mosaic-view of organism traits. Finally, the insistence in evaluating the evolution of snakes solely based on morphology and the fossil record should be seen as an unreasonable resistance to integrative approaches.

Future research may dissect the mechanisms linked with fast skull development in snakes, sexual shape dimorphisms, the microevolutionary dynamics linked with the early evolution of snakes, the specific type of deep terrestrial ancestor of snakes, the relationship between diet with skull shapes in snakes, and analyzing newly described and reconstructed fossils.

All that said, this dissertation opened new avenues and approaches to investigate snake and vertebrate evolution. Classical examinations of the relationship between form (shape), development (ontogeny), function (ecology), and evolution (phylogeny) such as “The Origin of Species” by Charles Darwin (1859), “On Growth and Form” by D’Arcy Thompson (1917), and “Ontogeny and Phylogeny” by Stephen Jay Gould (1977), have not included the evolution of the snake skull. This dissertation filled in that knowledge gap with intelligible, plausible, and fruitful outcomes.

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I apologize to those I did not mention. Last, fight for democracy and equality! Get vaccinated! Go out there and make Science diverse and fair!

CONTENTS

LIST OF CONTRIBUTED PUBLICATIONS

The following publications, all submitted and accepted in peer-reviewed journals, are contributed to this dissertation:

I: Filipe O. Da Silva, Anne-Claire Fabre, Yoland Savriama, Joni Ollonen, Kristin Mahlow, Anthony Herrel, Johannes Müller & Nicolas Di-Poï.The ecological origins of snakes as revealed by skull evolution. Nature Communications, 9, 376 (2018). https://doi.org/10.1038/s41467-017-02788-3

II: Joni Ollonen, **Filipe Oliveira Da Silva**, Kristin Mahlow, Nicolas Di-Poï (2018). Skull development, ossification pattern, and adult shape in the emerging lizard model organism *Pogona vitticeps*: A comparative analysis with other squamates. Frontiers in Physiology, 9. https://doi.org/10.3389/fphys.2018.00278

Author contributions:

I: **F.O.D.S**. and N.D.-P. designed the experimental approach. **F.O.D.S.**, K.M., and N.D.-P. selected the species sampling, and micro-CT scans were carried out by **F.O.D.S.**, K.M., J.O., and J.M. **F.O.D.S.** collected 2D and 3D landmark data. **F.O.D.S.**, A.-C.F., Y.S., A.H., J.O., and N.D.-P. performed the experiments. **F.O.D.S**., A.-C.F., Y.S., and N.D.-P. analyzed the data. **F.O.D.S.** and N.D.-P. collected and prepared some of the reptile embryos. **F.O.D.S.** and N.D.-P. prepared the figures and wrote the paper and all co-authors contributed in the form of discussion and critical comments. All authors approved the final version of the manuscript.

II: JO, **FD**, and ND-P: Designed the experimental approach; JO, KM, and **FD**: Performed the micro-CT scans; JO and **FD:** Collected 3D landmark data; JO and **FD**: Performed all other experiments; JO, **FD**, and ND-P: Analyzed the data; JO and ND-P: Collected and prepared the *Pogona vitticeps* embryos; JO and ND-P: Prepared the figures and wrote the paper; **FD** and KM: Contributed in the form of discussion and critical comments. All authors approved the final version of the manuscript.

1) INTRODUCTION

"(...) from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

Charles Darwin, 1859, *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*.

1.1) THE GROWTH OF SCIENTIFIC THOUGHT

François Jacob wrote in "Evolution and Tinkering" (1977):

> Whether mythic or scientific, the view of the world that man constructs is always largely a product of imagination. For the scientific process does not consist simply in observing, collecting data, and in deducing from them a theory. One can watch an object for years and never produce any observation of scientific interest. To produce a valuable observation, one has first to have an idea of what to observe, a preconception of what is possible. Scientific advances often come from uncovering a hitherto unseen aspect of things as a result, not so much of using a new instrument, but rather of looking at objects with a different angle. (p. 1161).

A scientist is aided by a combination of an imaginative scientific imagination anchored by conceptual thinking. A concept is a thought or notion that is conceived in the mind (Margolis & Laurence, 2007). Ernest Mayr (1982) saw biological concepts as central to the growth of biological thought.

Thomas Kuhn (1970) laid the theoretical grounds for the rise of Conceptual Change Theory (Posner et al., 1982). It posits that acceptance of new scientific concepts requires dissatisfaction with their anomalous versions. New concepts are embraced if intelligible (the learner understands it), plausible (it has explanatory power to solve misfits between expectation and observation), and fruitful (applicable to other fields).

Conceptual changes can culminate in scientific revolutions:

> Each of them [scientific revolutions] necessitated the community's rejection of one time-honored scientific theory in favor of another incompatible with it. Each produced a consequent shift in the problems available for scientific scrutiny and in the standards by which the profession determined what should count as an admissible problem or as a legitimate problem-solution. And each transformed the scientific

> imagination in ways that we shall ultimately need to describe as a transformation of the world within which scientific work was done. Such changes, together with the controversies that almost always accompany them, are the defining characteristics of scientific revolutions. (Kuhn, 1970, p. 6).

Scientific revolutions begin from a scientific crisis - a widespread lack of consensus about a "solution" to an old and complex scientific problem (Kuhn, 1970). A problem in biology that has been debated for more than a century is the nature, tempo, and mode of the ecological changes that took place in the early evolution of snakes from lizards (Bellairs & Underwood 1951, McDowell, 1972; Rieppel, 1988; Irish, 1989; Greene, 1997; Caldwell, 1999; Coates & Ruta, 2000; Greene & Cundall, 2000; Holman, 2000; Rieppel et al., 2003; Rage & Escuillié, 2003; Caldwell, 2007; Cundall & Irish 2008; Palci, 2014; Evans, 2015; Caldwell et al., 2019). Ecological hypotheses and evolutionary scenarios have coexisted, or even been recombined.

In biology, lack of consensus is recurrent and conceptual changes are harder to take place (Mayr, 1982; Kuhn, 1970). This is understandable based on the nature of the scientific inquiry. “On the contrary, it is just the incompleteness and imperfection of the existing data-theory fit that, at any time, define many of the puzzles that characterize normal science.” (Kuhn, 1970, p. 146). Normal science defines the notion of scientists and their ideas revolving around a current paradigm (Kuhn, 1970). In this sense, the ecological origin of snakes and evolution has been studied mostly from similar types of data for centuries (e.g., linear measurements, morphological descriptions, and phylogeny), so within the framework of normal science. That combination of data has advanced our understanding of snake evolution but remained with conflicting scenarios.

As expected, new sources of data, analytical approaches, and use of new technologies have been more recently sought to deal with this old and complex scientific problem (e.g., Scanferla & Bhullar, 2014; Werneburg & Sanchez-Villagra, 2015; Yi & Norell, 2015; Hsiang et al., 2015). Those studies, in addition to Bhullar et al. (2012) and Barros et al. (2011), have greatly inspired my approach. The research results and discussion described in the accompanying publications and this dissertation text can be understood to have deeply broken the pattern of normal science in the field of snake evolution because of its innovative and integrative design that produced well-supported results. State-of-the-art technologies and analytical procedures that are available to investigate geometric shapes were interconnected with large-scale phylogenetic, paleontological, ecological, and developmental data. A new ecological hypothesis and evolutionary scenario emerged regarding the early origin of snakes and their subsequent ecological radiation.

However, by no means the debate is over. It can be claimed that now we have an integrative foundation. Indeed, hardly so, consensus can be achieved in biology. An exception seems to be the theory of evolution (Darwin, 1859). Theodosius Dobzhansky (1973) wrote in his influential publication "Nothing in biology makes sense except in the light of evolution that “Seen in the light of evolution, biology is, perhaps, intellectually the most satisfying and inspiring science. Without that light, it becomes a pile of sundry facts-some of them interesting or curious but making no meaningful picture as a whole." (p.129).

Although consensus does take a long time to be achieved, if ever, refutability is more commonplace. The falsification principle states that hypotheses cannot be guaranteed status of eternal and immutable acceptance but should be stated so that they could be falsified (Popper, 1959).

Karl Popper (1959) dissected falsifiability in “The Logic of Scientific Discovery”:

> I shall certainly admit a system as empirical or scientific only if it is capable of being *tested* by experience. These considerations suggest that not the *verifiability* but the *falsifiability* of a system is to be taken as a criterion of demarcation. In other words: I shall not require of a scientific system that it shall be able to be singled out, once and for all, in a positive sense; but I shall require that its logical form shall be such that it can be singled out, by means of empirical tests, in a negative sense: *it must be possible for an empirical scientific system to be refuted by experience.* (p. 18).

We can now access scientific hypotheses using modern statistics. Hypotheses that fall out of a demarcation point are rejected. P-values became the norm of demarcation following the rise of statistics in the early 20th century (Salsburg, 2001). It is though currently under debate if the p-value can be used in isolation or if other metrics need to be employed all along (Altman & Krzywinski, 2017; Amrhein et al., 2019). Complementary metrics are indeed being suggested (Halsey et al., 2019). This debate is though still ongoing, and the p-value continues being accepted as a key statistical metric for biologists. In this dissertation, biological comparisons and tests of hypotheses relied on mathematical and statistical formalizations that generate p-values, but it is also supported by a variety of other independent sources of evidence.

In an attempt to unify those two approaches, Kuhn (1970) wrote:

> Furthermore, it is in that joint verification-falsification process that the probabilist’s comparison of theories plays a central role. Such a two-stage formulation has, I think, the virtue of great verisimilitude, and it may also enable us to begin explaining the role of agreement (or disagreement) between fact and theory in the verification process. (...) It makes a great

deal of sense to ask which of two actual and competing theories fits the facts better. (p. 147).

Popper (1959) stated something alike but from a Darwinian framework that "Its aim [empirical method] is not to save the lives of untenable systems but, on the contrary, to select the one which by comparison is the fittest, by exposing them all to the fiercest struggle for survival." (p. 20).

In sum, in the philosophical spirit described above, the field of snake evolution undergoes a scientific crisis. New types of data, technologies, and integrative approaches to address complex problems tend to pay off. Thus, this dissertation aimed at producing the first large-scale and integrative macroevolutionary study of the skull shape evolution of snakes and lizards. It addressed the old and complex problem of the ecological origin and radiation of snakes. I proposed a new ecological hypothesis (terrestrial-to-fossorial scenario), rejected previously proposed hypotheses on snake origins, and pointed out some major conceptual flaws present in the field of snake evolution (e.g., misinterpretation of phylogenies and fossils).

To achieve a full appreciation of the problem dealt herein, in the following sessions, I introduced the general biology of lizards and snakes, reviewed the history of the problem, and showcased the previous use of geometric morphometrics in squamates - an approach capable of capturing quantitative variation (shape and size = form). It was *a priori* envisioned during the research included in this dissertation as having great potential to generate new insights regarding the ecological origin and radiation of snakes.

1.2) SQUAMATA: LIZARDS AND SNAKES

Figure 1 shows a phylogeny of extent taxonomic groups and relevant fossils. Centuries of morphological and phylogenetic studies demonstrated that snakes evolved from lizards (Pough et al., 2004; Cundall & Irish 2008). Snakes are then specialized lizards (Greene, 1997). I adopted the terminology of lizards and snakes for simplicity. They form the Squamata - a diapsid Order with species differing dramatically in their skull temporal region (Oppel, 1811); and with Rhynchocephalia (*Sphenodon* and fossil relatives) form Lepidosauria (Evans, 2003).

Dated phylogenies in combination with the fossil record indicate that lepidosaurs might have appeared before the Permian/Triassic extinction event (Irisarri et al., 2017; Simões et al., 2018, 2020), and diversified in the Triassic (Simões et al., 2020; Evans 2003). Squamates might have appeared near the Permian/Triassic boundary and diversified in the Upper Triassic (Irisarri et al., 2017; Simões et al., 2018, 2020), or they might have originated in the Lower Jurassic (Burbrink et al., 2020). Snakes most likely appeared in the Jurassic (Garberoglio et al., 2019; Harrington & Reeder

2017; Caldwell et al., 2015,) and crown snakes in the Lower Cretaceous (Harrington & Reeder 2017; Burbrink et al., 2020). Yet, estimations have yielded different means and ranges, interpretations are then contentious (Jones et al., 2013).

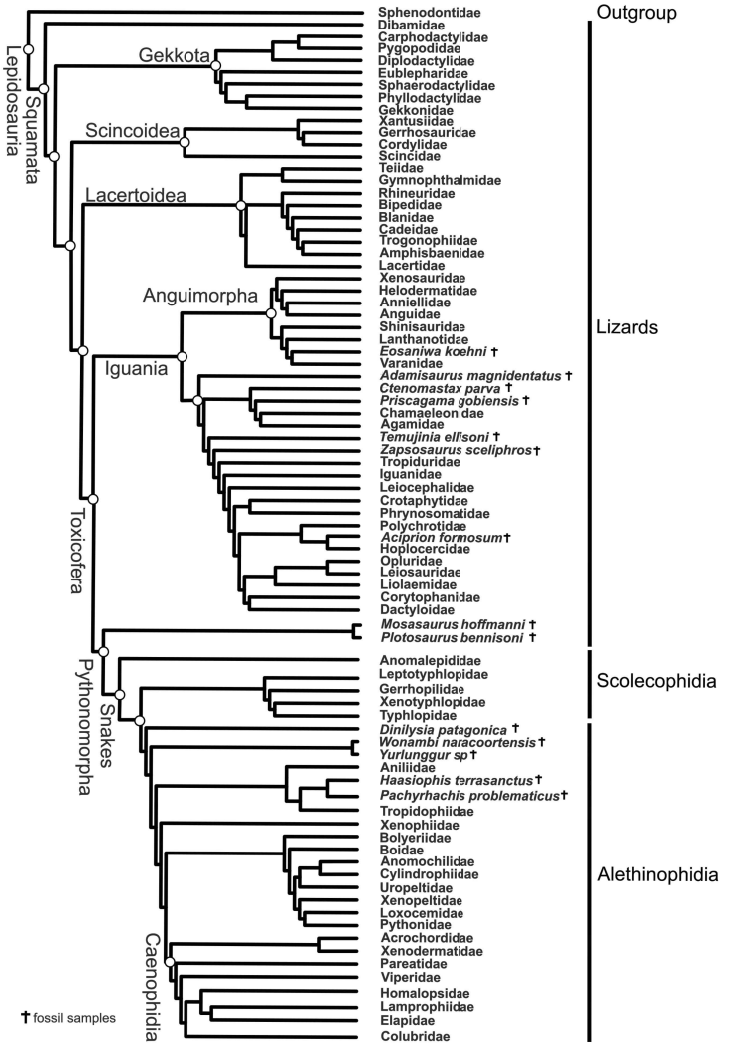

Figure 1 A simplified phylogeny of lizards and snakes. Snakes (Ophidia), Iguania, and Anguimorpha form Toxicofera - supported by molecular and combined inferences (Vidal & Hedges, 2005; Pyron et al., 2013; Reeder et al., 2015). M*osasauroids* and snakes form Pythonomorpha (Cope, 1869; Reeder et al., 2015). Snakes are subdivided into likely paraphyletic Scolecophidia (Cope, 1864; Underwood, 1967) - composed of miniaturized fossorial species (Pyron et al., 2013; Miralles et al., 2018); and the mcnophyletic Alethinophidia (Nopcsa, 1923; Pyron et al., 2013) - snakes that most people have in mind. Caenophidian is monophyletic (Underwood, 1967) - it includes, among others, the so feared vipers.

Rhynchocephalians have likely persisted in Gondwana until the end of the Cretaceous while went extinct in Laurasia, only to be found nowadays in New Zealand (Apesteguia & Novas, 2003). Lizards were interpreted to have already played a major ecological role at that time in Gondwana (Simões et al., 2015b). Snakes might have radiated in Gondwana whereas lizards in Laurasia (Martill et al., 2015; Simões et al., 2015b). It is also debated if snakes originated in Laurasia or Gondwana (Hsiang et al., 2015; Martill et al., 2015). Currently, squamates have a global distribution but in the polar regions (Pianka & Vitt, 2003; Uetz & Hošek, 2020).

The diversity of lizards and snakes is astonishing, extant lizards and snakes are represented by 7K and 3.8K species, respectively (Uetz & Hošek 2020; see alternative estimates in Wallach et al., 2014). They display striking diversity in almost every aspect of their biology (Greene, 1997; Pianka & Vitt, 2003). For example, multiple evolutionary origins and convergences took place concerning their body length-shape, limb-digit reduction, venom, parthenogenesis, and mode of reproduction (Wiens & Slingluff, 2001; Wiens et al., 2006; Losos et al., 2009; Kearney et al., 2009; Kohlsdorf et al., 2010; Sites et al., 2011; Pyron & Burbrink, 2014).

1.2.1) THE SNAKE BODIES

As a research trend, more focus has been given to integrating results for the postcranial skeleton, meaning that an integrative picture of vertebral development with ecomorphologies and qualitative-quantitative phenotypic evolution is better defined than for the skull.

Snakes have long bodies linked with a 'clock-and-wavefront' mechanism in which higher rates of somitogenesis generate more and smaller somites for a longer time (Gomez et al., 2008). Somites also differentiate into vertebrae and so snakes have a higher number of them than lizards (Müller et al., 2010). Snakes have also shorter caudal and cervical regions whereas an elongated thoracic region in comparison to lizards (Müller et al., 2010). This peculiar pattern has been associated with the homogenization of *Hox* gene expression domains (Cohn & Tickle, 1999) and retention of the standard vertebrate *Hox* domains of expression but with downstream regulatory alterations (Di-Poï et al., 2010; Woltering, 2012; Guerreiro et al., 2013). The body shapes of sea snakes seem to diverge by both mechanisms (Sherratt et al., 2019a). Moreover, snakes would have lost most of their axial regionalization (Cohn & Tickle, 1999), but the comparison of vertebral shapes through geometric morphometrics in comparison to *Hox* gene expression domains suggests that it has been at least partially retained (Head & Polly, 2015).

The size and qualitative morphology of vertebrae in snakes can also provide indications of snake ecologies. Small vertebrae with low neural arches are associated with fossoriality (e.g., Martill et al., 2015). Laterally compressed bodies with heavily ossified vertebrae and ribs (pachyostosis)

indicate marine habitats (e.g., Scanlon et al., 1999). Moreover, vertebrae of snake fossils, for example, the enormous *Titanoboa*, have been used to reconstruct paleoclimates and indicated global warming in the Palaeocene neotropics (Head et al., 2009). Furthermore, ecomorphs of lizards and snakes are distinguishable from linear measurements and ratios of their bodies (Wiens et al., 2006; Müller et al., 2011; Grizante et al., 2012; Losos et al., 2009; Lee et al., 2016; Moon et al., 2019). *Anolis* emerged as a model organism for eco-evo-devo due to those correlations of form and function (Losos, 2009).

Most lizards have fore- and/or hindlimbs. Some species of *Bachia* are an exception because they have only forelimbs. Recurrent evolution of limbless species happened in all major lineages, except for Iguania (Brandley et al., 2008; Evans, 2015). Snakes lack forelimbs in all living or fossil species (Brandley et al., 2008), but for the putative four-limbed fossil *Tetrapodophis* (Martill et al., 2015). Some extant snakes (e.g., pythons, boids, and blind snakes) have vestigial hindlimbs (Leal & Cohn, 2016). Several snake fossils had different numbers of hindlimb elements such as *Pachyrhachis, Eupodophis*, *Haasiophis*, and *Najash* (Zaher et al., 2009; Palci et al., 2013a, b). The loss of limbs correlates with body elongation (Wiens et al., 2006), fossoriality or sand-swimming behavior (Macaluso et al., 2019), changes in the structure and/or domain of expression of *Hox* genes (Cohn & Tickle, 1999; Di-Poï et al., 2010), as well as modifications of regulatory sequences with arresting of limb development (Kvon et al., 2016; Leal & Cohn, 2016; Roscito et al., 2018). Furthermore, changes in limb proportions can evolve quickly, for example, due to unexpected events such as hurricanes (Donihue et al., 2018). Finally, the dynamic pattern of digit loss and gain has been shown for lizards to break Dollo's law (Brandley et al., 2008; Kohlsdorf et al., 2010). *Tetrapodophis* and *Haasiophis* are the only known snakes to have preserved digits (Martill et al., 2015; Tchernov et al., 2000; Lee et al., 2016).

1.2.2) THE SNAKE SKULLS

The skull of lizards and snakes have been extensively described in qualitative terms and integrated mostly with phylogenetic and taxonomic studies (Rieppel, 1993a; Evans, 2008; McDowell, 2008; Cundall & Irish, 2008; Gans & Montero, 2008). Figure 2 shows a few skulls, a tiny but representative fraction of the variation seen in ~11K species of squamates.

More attention has been given to the relationship between skull phenotype and diet. Habitats and development received much less attention.

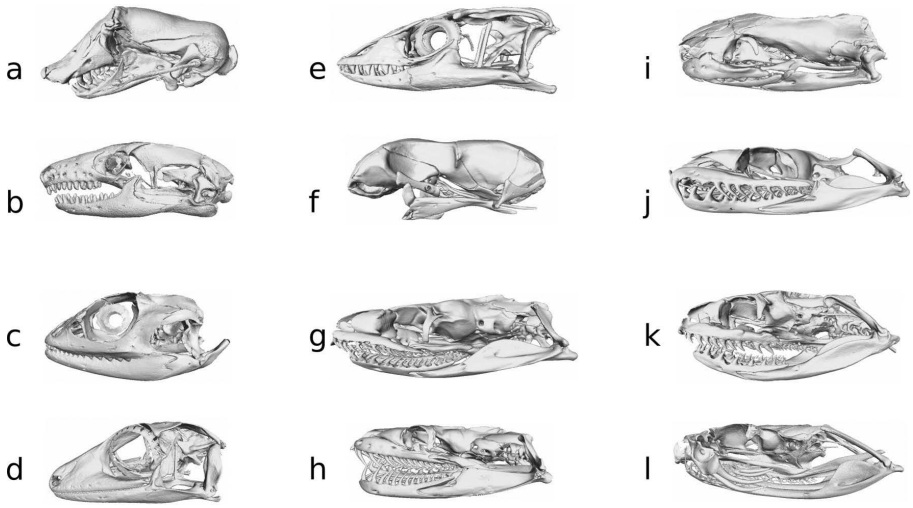

Figure 2 Morphological diversity in the skull of lizards: a - *Leposternon*, b - *Acontias*, c - *Iguana*, d - *Aeluroscalabotes*, e - *Varanus;* and snakes f - *Letheobia* (Scolecophidia), g - *Tropidophis*, h - *Python*, i - *Cylindrophis*, j - *Boa*, k - *Boaedon*, l - *Bitis.* Lizards and snakes variably lost elements of their temporal region and other skull parts. Reacquisitions have occurred, for instance, for the lower temporal bar in fossils (Simões et al., 2016b). The snake skulls show a bone-enclosed brain (partially convergent with fossorial lizards, for instance, amphisbaenians), higher flexibility and mobility of bone and skull parts (at variable degrees and to some extent convergent with lizards, e.g., geckos), a larger gape size (at variables degrees, for instance, substantial differences exist between "scolecophidians" and alethinophidian snakes. Absence of the epipterygoid (linked with reduction of the chondrocranium) and loss of the temporal bars as well as fenestras (see also for lizards e.g., amphisbaenians) (Rieppel, 1993; Evans, 2008; McDowell, 2008; Cundall & Irish, 2008; Gans and Montero, 2008). Curiously, snakes lack external ears but have a hearing mechanism based on transmit ground-borne vibrations (Christensen et al., 2012). The lack of tympanic membrane/cavity is seen, for example, also in chameleons (Wever, 1968).

Skull bone conformation and size are linked with lizard diets (Metzger & Herrel, 2005; Herrel, 2007; Kohlsdorf, 2008). Herbivory has exclusively, but rarely, evolved in lizards (most of them are insectivorous) (Espinoza et al., 2004). Furthermore, lizards evolved more tooth types than snakes (multicuspid and crested) that benefit in their exploitation of variable diets, including eating plants (Zahradnicek et al., 2014). Snakes are carnivorous and prey on diverse animal sizes by whole-body ingestion - from insect larvae to large mammals (Greene, 1983; Moon et al., 2019). Food items, particularly in snakes, are dependent on the whole skull

features (size, shape, and gape size) but also habitat, geographical distribution, sex, constriction, and presence of fangs with venom glands (Moon et al., 2019).

Interestingly, new feeding mechanisms evolved in snakes like mandibular racking (Kley & Brainerd, 1999), the differential function of asymmetric maxillae (Hoso et al., 2010), mandibular sawing (Kojima et al., 2020), specialized fangs (Vonk et al., 2008), independent movements (left and right palatine, pterygoid, maxilla), larger gape size, and constriction (McDowell, 2008; Cundall & Irish, 2008; Moon et al., 2019). Venom delivery systems have evolved higher complexity within helodermatid lizards and colubroid snakes (Sites et al., 2011), while venom itself is understood to have had a single early origin in Squamata (Fry et al., 2006). Rear and front fags are homologous based on gene expression and ontogenetic allometric patterns of the maxilla and pterygoid bones (Vonk et al., 2008).

Regarding habitat, variable degrees of fossoriality are associated with skull miniaturization, conformational changes, and loss of bones in lizards and snakes (Rieppel, 1984; Gans & Montero, 2008; Barros et al., 2011). Loss of skull bones is a common trend in Squamata (Rieppel & Gronowski, 1981; Herrel et al., 2007; Evans, 2008; McDowell, 2008; Cundall & Irish, 2008). In fact, most likely product of convergence (Figure 2). The relationship between habitat and skull morphology became clear after quantification and analysis of skull shape with geometric morphometrics (see section 1.5).

Regarding the skull development, lizards and snakes have been mostly described based on the chondrocranium (Bellairs & Kamal, 1981; Diaz & Trainor, 2019). Skull bones in other vertebrates, presumably also for snakes and lizards, are mostly dermal (dermatocranium: e.g., premaxilla), but some are endochondral (neurocranium, for instance, braincase; splanchnocranium, for instance, quadrate) (Helms & Schneider, 2003; Piekarski et al., 2014). Most bones in the skull originate from neural crest cells (Abzhanov et al., 2007; Piekarski et al., 2014), but, for example, the parietal and frontal bones vary among vertebrates (neural crest and/or mesoderm cells) (Piekarski et al., 2014; Maddin et al., 2016). The origin of the cells of the neural crest (e.g., mandibular, hyoid, etc.) making up the same homologous skull bones can also vary among vertebrates, which has been pointed out as an example of 'developmental system drift', in which interspecific divergence in developmental processes that underlie homologous characters occurs with little or no change in the adult phenotype (Piekarski et al., 2014). Cranial neural-crest cell fate mapping remains to be undertaken in lizards and snakes. There is also little information about the development of the dermal skull bones in lizards and snakes in terms of mechanisms.

In terms of known developmental processes, the onset of ossification differs between lineages, for example, for the snout bones (Werneburg &

Sánchez-Villagra, 2015). Moreover, the frontal and parietal are known to be the last bones to complete ossification in the postnatal development of lizards (Maisano, 2001). Additionally, it was confirmed in a large-scale study that the frontal-parietal suture corresponds to the separation of the fore-midbrain frontier throughout the skull development of "reptiles", indicating a close association between ossification of the skull rooftop and brain development (Fabbri et al., 2017). The oestrogen pathway underlies the evolution of exaggerated male cranial shapes in *Anolis* (Sanger et al., 2014).

Studies of embryonic skull bone development have been mostly focused on qualitative descriptions of ossification series for making staging tables, offering limited information on evolutionary processes and developmental mechanisms. Among lizards, total or partial embryonic description of the morphology and ossification levels of the skull bones are available for Chamaeleonidae: *Chamaeleo hoehnelii* (Rieppel, 1993b); Gymnophthalmidae: *Nothobachia ablephara* (Roscito & Rodrigues, 2012), *Calyptommatus sinebrachiatus* (Roscito & Rodrigues, 2012), *Vanzosaura rubricauda* (Roscito, 2010), *Ptychoglossus bicolor* (Hernández-Jaimes et al., 2012); Gekkonidae: *Cyrtodactylus pubisulcus* (Rieppel, 1992a), *Tarentola annularis* (Khannoon & Evans, 2020); Iguanidae: *Iguana iguana* (Lima, 2015); Polychrotidae: *Polychrus acutirostris* (Alvarez et al., 2005). Leiosauridae: *Anisolepis longicauda* (Guerra-Fuentes, 2006), Liolaemidae: *Liolaemus scapularis* (Lobo et al., 1995), *Liolaemus quilmes* (Abdala et al., 1997), Scincidae: *Liopholis whitii* (Hugi et al., 2010), *Acontias meleagris* (Brock, 1941), *Mabuya capensis* (Jerez, 2015); Tropiduridae: *Tropidurus sp.* (Guerra-Fuentes, 2006); Varanidae: *Varanus panoptes* (Werneburg et al., 2015); Lacertidae: *Lacerta agilis* (Rieppel, 1994), *Zootoca vivipara* (Rieppel, 1992b), Teiidae: *Salvator merianae* (Arias & Lobo, 2006). Recently, one fossil embryo of an Anguimorpha lizard with a well-preserved skull was described (Fernandez et al., 2015).

Among snakes, such studies are available for Acrochordidae: *Acrochordus granulatus* (Rieppel & Zaher, 2001); Elapidae: *Naja kaouthia* (Jackson, 2002), *Naja haje* (Khannoon & Evans, 2015); Pythonidae: *Python sebae* (Boughner et al., 2007), Lamprophiidae: *Psammophis sibilans* (Al Mohammadi et al., 2019; Kamal & Hammouda, 1965), *Pantherophis alleghaniensis* (described in the publication as *Elaphe obsoleta*) (Haluska & Alberch, 1983), *Boaedon fuliginosus* (Boback et al., 2012); Colubridae: *Crotaphopeltis hotamboia* (Brock, 1929), *Natrix natrix* (Sheverdyukova, 2017, 2019), *Helicops leopardinus* (Di Pietro et al., 2014); Viperidae: *Bothropoides jararaca* (Polachowski & Werneburg, 2013); *Cerastes cerastes* (Khannoon et al., 2020); and one snake fossil embryo although without its skull, the Cenomanian forest-dwelling snake *Xiaophis*, was reported (Xing et al., 2018).

As exceptions to purely intra-specific descriptive approaches, snakes and lizards have been shown to have the species-specific ordering of

external morphological traits, making macroevolutionary comparisons based on staging tables impracticable or, at least, very challenging (Andrews et al., 2013). External traits used to define embryonic stages have been shown to have a limited phylogenetic signal (Skawiński & Borczyk, 2017). Even the phylotypic stage, which is considered a trademark of vertebrate evolution, has been called out based on close-up examination of external morphology (Richardson et al., 1997). Conversely, a phylotypic period has been supported, for example, by transcriptomes (Irie & Kuratani, 2011). Objections to macroevolutionary comparisons of embryos based on discrete stages have been raised, suggesting that comparisons between embryos should focus on causal traits (Alberch, 1985). From this point of view, one could perform comparisons of phenotypic skull changes such as shape and other causal features (e.g., sequential closure of pharyngeal slits and growth of the maxillary prominence) in embryos soon after their oviposition, hatchlings, and mature adults.

In sum, previous to the publications included in this dissertation, the skull lacked a macroevolutionary integrative approach that could unite morphology, ecology, paleontology, phylogeny, functional morphology and the development of lizards and snakes. This also hampered our understanding of the early ecology of snakes and snake evolution as a whole. Following, I reviewed studies on this problem.

1.3) A REVIEW ON THE ECOLOGICAL ORIGIN OF SNAKES

The ecological origin of snakes is one of the most controversial topics in vertebrate evolution (Bellairs & Underwood, 1951; McDowell, 1972; Rieppel, 1988; Irish, 1989; Caldwell, 1999; Coates & Ruta, 2000; Greene & Cundall, 2000; Holman, 2000; Rieppel et al., 2003; Rage & Escuillié, 2003; Caldwell, 2007; Cundall & Irish, 2008; Palci, 2014; Evans, 2015; Caldwell et al., 2019).

Posed ecological hypotheses based on modern phylogenetic methods are terrestrial with fossorial behaviour (Forstner et al., 1995; Heise et al., 1995; Wiens et al., 2012; Longrich et al., 2012; Martill et al., 2015; Reeder et al., 2015); terrestrial but with uncertain behaviour (Vidal & Hedges, 2004; Apesteguía & Zaher, 2006; Conrad, 2008; Zaher & Scanferla, 2012); aquatic (but likely meant marine based on later publications by the same authors) (Caldwell & Lee, 1997; Caldwell, 1999); marine or semi-aquatic (Lee & Caldwell, 2000; Lee & Scanlon, 2002b); marine (Lee et al., 1999a; Caldwell, 2000; Rage & Escuillié, 2003; Lee, 2005a,b; Lee, 2009); marine or terrestrial (Scanlon et al., 1999; Lee, 1999b); marine or terrestrial (but non-fossorial) (Scanlon & Lee, 2000); marine and terrestrial (Caldwell et al., 2015); marine for the most recent common ancestor of all extinct and extant snakes (maybe fossorial for the crown snake ancestor) (Lee & Scanlon, 2002a); semi-aquatic in coastal environments (Caldwell et al.,

2019); terrestrial or fossorial (perhaps semi-fossorial) (Hsiang et al., 2015); non-fossorial (Palci et al., 2013a); non-marine (Wiens et al., 2010); unclear (Tchernov et al., 2000; Palci et al., 2013b; Werneburg & Sánchez-Villagra, 2015; Lee et al., 2016).

1.3.1) THE HISTORY OF THE DEBATE

The history of this heated debate dates to the 19th century and some of the methodological and conceptual problems that confused the debate at the time persist today. Initially, the marine ecology of early snakes was interpreted by taking into consideration the identified close relationship between large marine lizards (e.g., mosasaurs) and snakes (Nopcsa, 1908, 1923). This conclusion stemmed from earlier findings of a Pythonomorpha clade (Cope, 1869), and its historical debate (Owen, 1877; Cope, 1878; Caldwell, 1999). Cope (1869) has often been associated with the birth of the marine hypothesis, but he held a different view "that terrestrial representatives now unknown to us, inhabited the forests and swamps of the Mesozoic continents, and strove for mastery with the huge dinosaurs, that also sought their shades, is probable". Marine fossil lizards were proposed at the time (and resurfaced by some contemporary authors) as the "perfect" transitions between lizards and snakes, implying that snakes would have evolved from a marine ancestor.

This limited deduction generated a heated debate. Alternative hypotheses soon emerged at the time. A terrestrial ancestor that inhabited areas with thick vegetation and perhaps had a semi-fossorial behavior (Janensch, 1906); a terrestrial, grass-living, anguimorphid lizard (Camp, 1923); or a fossorial ancestor (Mahendra, 1938).

Eye anatomy (Walls, 1940,1942; see also recent studies for more complex views on that matter; Simões et al., 2015a; Simões et al., 2016a; Caprette et al., 2004) and other anatomical sources also seemly supported a fossorial origin (Bellairs & Underwood, 1951; Underwood, 1967; Underwood, 1970, Senn & Northcutt, 1973). However, views continued partially differing: a fossorial or marine (McDowell, 1972), a secretive (perhaps semi-fossorial) (Rieppel, 1978), and non-marine ancestors (Haas, 1980a,b). The fossorial origin of snakes became the most accepted over time, meaning that fossorial lizards would have given rise to fossorial snakes (Rieppel, 1988; Irish, 1989; Forstner et al., 1995; Heise et al., 1995; Kardong et al., 1997; Greene, 1997).

That was about to change when mosasaurs were found again to be the sister group of snakes in a modern phylogeny, although that did not lead first to an explicit suggestion of a marine origin of snakes (Lee, 1997), it did become quickly so after the redescription of the marine fossil snake *Pachyrhachis problematicus* (Caldwell & Lee, 1997). Thus, an aquatic or marine origin of snakes was resurfaced solely supported by the interpretation of the sister-group position of this fossil concerning all other

snakes (Caldwell & Lee, 1997; Lee, 1998; Lee & Caldwell, 1998).

Those findings were met with criticism but also quick acceptance. In a reanalysis of the fossil, *Pachyrhachis* was recovered nested in crown snakes (Zaher, 1998). His criticism was focused on what was for the author, the unreasonable definition of characters, and the exclusion of *Dinilysia patagonica* (a well-known terrestrial fossil already at the time) for no explicit reasons. On the other hand, the redescription and phylogenetic treatment of another marine fossil snake *Pachyophis woodwardi* supported it as the sister species, and together with *Pachyrhachis,* of all the other snakes (Lee et al., 1999b). This time, however, a marine or a terrestrial origin of snakes were considered equally parsimonious. This was also concluded after a paleoecological reexamination of *Pachyrhachis* in the context of bed sediments and paleofauna (Scanlon et al., 1999). *Pachyrhachis,* despite further criticism (Zaher & Rieppel, 1999), became quickly known as a "well-preserved transitional taxon" (Scanlon et al., 1999; Lee et al., 1999b). Additionally, mosasaurs continued being stated as an "intermediate stage", so that it was used to claim a marine origin of snakes and polarize the evolution of the ophidian feeding mechanism (Lee et al., 1999a).

Early on, it becomes apparent that the same authors were reaching variable conclusions about the early ecology of snakes after examining the same fossils. Surely, the debate intensified about sample size, interpretation of anatomical features, the definition of characters, presence of homoplasy, and trait correlations out of important incongruences (Lee, 1998; Zaher & Rieppel, 1999; Zaher & Rieppel, 2000).

Igniting, even more, the polemics, the description of a new marine fossil snake, *Haasiophis terrasantus*, and its phylogenetic analysis based on a new morphological matrix, showed that it was nested, and together with *Pachyrhachis,* within crown snakes (Tchernov et al., 2000). These two marine fossil snakes were then considered by those authors to "have no particular bearing on snake-mosasauroid relationships or snake origins." (Tchernov et al., 2000). The debate simply intensified but continued to be restricted to morphology and phylogeny (Zaher & Rieppel, 2000; Rieppel & Zaher, 2000; Lee & Caldwell, 2000).

The redescription of the fossil snake *Wonambi naracoortensis,* which was done together with the new description of skull parts of *W. Barriei,* supported the position of *Pachyrhachis and Pachyophis* as stem-snakes, and more inclusively, *Wonambi* itself (Scanlon & Lee, 2000). Again, a large marine or terrestrial ancestor of snakes were both considered likely, but authors explicitly excluded a fossorial origin.

The description of *Eupodophis descouensi*, and its inclusion in a phylogenetic framework, recovered the marine fossil snakes either as a clade that is sister to all snakes or individually branched out as stem species (Rage & Escuillié, 2000). Those authors suggested that "the macrostomatan condition of the skull more likely represents the primitive condition within

Ophidia" (p. 515), without discussing its ecology.

Eventually, Caldwell (2000) replied to the criticisms of Zaher (1998). He ratified character definitions and modified others but criticized Zaher (1998) for not having provided a data matrix for reexamination. Interestingly, Caldwell (2000) wrote in his reply that "a marine origin for snakes is now a reasonable alternative to the received position that snakes originated from a burrowing or fossorial ancestor" (p. 189). In support of his assertion, he cited Lee (1997), Caldwell and Lee (1997), Caldwell (1999), and Lee and Caldwell (1998). Surprisingly, Lee (1997) in addition to Lee and Caldwell (1998) did not explicitly discuss the marine origin of snakes, as both works were focused on the phylogenetic position of marine fossil lizards. Thus, implicitly, snakes were seen in those studies as having a marine origin.

In response to Caldwell (2000) as well as Scanlon and Lee (2000), reanalyses of the marine fossil snakes by Zaher and Rieppel (2002) in addition to *Wonambi* by Rieppel et al. (2002), have both recovered a nested position of those fossils inside crown snakes. They interpreted those fossils available held little information about the ecological origin of snakes and were an indication that a large snake ancestor was unlikely to be the case.

Furthermore, Zaher and Rieppel (2002) incisively criticized the research approach by Caldwell (2000), which for them consisted of reanalyzing Zaher (1998) based on Caldwell's own interpretation of the coding of characters. Second, they pointed out that "there exists no rational basis for excluding *Dinilysia* from any analysis of higher-level interrelationships of snakes, especially as relationships of fossil snakes are addressed" (p. 105).

Finally, Rieppel et al. (2002) commented:

> problems of character coding for madtsoiids, based primarily on *Wonambi*, are related to the referral of fully disarticulated material to this taxon, and to inferences about missing elements drawn from neighboring bones. The basis for inferences about missing elements may furthermore be prejudiced by a priori hypotheses of relationships. (p. 813).

Ultimately, Zaher and Rieppel debated choices of character coding, acknowledging changes while reaffirming others. Thus, one can see that improvements were being made in the morphological data matrices out of these debates and an increasing number of snake fossils were being described, but the ecology of early snakes remained troublesome. Lee and Scanlon (2002a) reanalyzed the phylogeny of squamates and recovered again that *Pachyrhachis*, *Pachyophis*, and *Haasiophis* were sister species of all the other snakes, proposing then a non-fossorial origin of snakes and macrostomy as the ancestral state.

Criticism of the phylogenetic position of *Pachyrhachis* as the sister species of snakes was summarized after a complete anatomical description

of *Haasiophis* regarding other snakes (Rieppel et al., 2003). These authors claimed arbitrary down weighting of characters linked with fossoriality, disregard of developmental data (e.g., interpretation of snakes having an extended neck instead of a thoracic region), differential interpretations of anatomical features for the same specimens, inconsistent definition of homologous structures, and exclusion of relevant fossil taxa. They highlighted that the ecology of early snakes was not possible to be sorted out with confidence from fossils and the phylogenetic hypotheses available at the time. Instead, Rieppel et al. (2003) suggested that molecular phylogenies would be key to address the problem and circumvent convergence (homoplasy).

Different from expectation, molecular phylogenies led to distinct ecological propositions: fossorial (Forstner et al., 1995; Wiens et al., 2012) or terrestrial (but is unclear if it meant terrestrial with fossorial behavior) (Vidal & Hedges, 2004). A marine origin of snakes was suggested to be supported by molecular data, but the molecular phylogeny of Vidal and Hedges (2004) was instead only used to constrain the morphological data and so a new molecular phylogeny had not been produced (Lee, 2005a).

Later, Scanlon (2005) amended anatomical interpretations for *Wonambi* based on new fossil findings. Without a phylogenetic treatment, this author defended its stem position together with marine fossils versus the topology estimated by Rieppel et al. (2002). Improving his assertion, with the description of the non-marine snake fossil *Yurlunggur sp*., he found that it was grouped with *Wonamb*i (Scanlon, 2006), and, in turn, they formed the sister clade of the clade composed by *Dinilysia* + crown snakes. The marine fossil snakes were found as the sister species of all the other snakes. The ecology of this new fossil and the snake ancestors were not discussed, perhaps indicating his perception that those fossils would offer limited resolution about the early ecology of snakes.

On the other hand, the description of the snake fossil *Najash rionegrina*, interpreted to have a subterranean or surface-dwelling lifestyle, was initially considered to have significantly overturned previous ecological and phylogenetic findings (Apesteguia & Zaher, 2006; Zaher et al., 2009). *Najash* was found to be the sister species of all the other extant and extinct snakes. *Dinilysia*, the marine fossil snakes, and *Wonambi* were all found nested inside crown snakes. Those authors suggested a terrestrial origin of snakes. However, after the description of new fossil remains of *Dinilysia*, those findings were met with criticism about the way that *Najash* characters were described, which were based on disarticulated samples from different localities (Caldwell & Calvo, 2008). Caldwell and Calvo (2008) went on to suggest that *Najash* would be instead found to be grouped with *Dinilysia* in future phylogenetic analyses. This type of prediction without analyses had been previously criticized as potentially reflecting research bias (Rieppel et al., 2002).

It became clear that analyzing small-scale morphological or

molecular features in isolation, even with new fossil findings, would not be enough to provide a clear ecological scenario for the early origin of snakes. Thus, the first combined molecular and morphological phylogeny emerged, in which it was found that the marine pachyophiids, *Dinilysia*, and *Wonambi* were the sister species to all living snakes (Lee et al., 2007). However, *Najash* and *Yurlunggur* were not included. Lee (2009), in a subsequent combined phylogenetic analysis, recovered *Haasiophis* and *Pachyrhachis* again as the sister species of the other snakes. Also, all snakes were nested inside a clade of large marine lizards, which was seen as indicative of a marine origin of snakes.

In opposition, a large-scale phylogenetic tree based on morphological characters recovered all above mentioned fossils nested inside crown snakes and the ancestor was interpreted to be terrestrial based on the close relationship between snakes with burrowing lizards (Conrad, 2008). However, *Najash* was not analyzed but that was acknowledged in his monograph. In agreement, a combined phylogenetic inference with new molecular and published morphological data (Conrad, 2008) recovered again *Dinilysia*, *Wonambi*, and Pachyophiidae snakes nested inside of crown snakes (Wiens et al., 2010).

Wiens et al. (2010) rejected the marine hypothesis and went on to criticize that:

> Lee (2009) assumed that the marine fossil snakes *Haasiophis* and *Pachyrachis* are outside of a clade formed by other snakes (i.e., snakes exclusive of these two genera were treated as a single terminal taxon, such that no analysis can contradict this assumption). (p. 686).

Departing from the traditional discussions of the early habitat ecology of snakes, the description of the terrestrial fossil snake, *Sanajeh indicus,* demonstrated that snakes could feed on hatchling sauropod dinosaurs already in the Upper Cretaceous (Wilson et al., 2010). Phylogenetic analysis showed that *Sanajeh, Dinilysia*, madtsoiid snakes, pachyophiid snakes, and *Najash* were all nested within crown snakes.

The redescription of the fossorial snake fossil *Coniophis precedens*, previously exclusively known from vertebral remains, but now including also skull fragments, and its inclusion in the snake phylogeny, yielded it as the sister species of all extant and extinct snakes (Longrich et al., 2012). All the other fossil snakes, except for *Najash*, were found nested within Alethinophidia. These authors interpreted this topology as supportive of a fossorial origin. At this point, the marine hypothesis had already become less accepted by most of the scientific community.

Furthermore, Zaher and Scanferla (2012) described new fossil findings for *Dinilysia* and a new phylogenetic analysis showed it as the sister taxon of all extant snakes. *Najash* was recovered as the first evolutionary offshoot in the snake phylogeny. *Yurlunggur*, *Haasiophis*,

Eupodophis, *Pachyrhachis*, and *Wonambi* were all found nested in crown snakes. The authors suggested a terrestrial origin of snakes and their results rejected the proposition by Caldwell and Calvo (2008) that *Dinilysia* and *Najash* would be found grouped.

Another large-scale morphological tree, which included CT-scanned samples, did not recover the same topology as the molecular data, and convergence was discussed as an important issue for squamate phylogenies (Gauthier et al., 2012). *Najash* and *Dinilysia* were found as the sister species of all the other snakes, while Simoliophiidae were found nested inside the crown-snake clade. The authors partially reviewed studies on snake evolution but did not express any conclusion on the ecology of early snakes, perhaps sensing those tree topologies and their relativist fossil placements were not enough to address this old and complex problem. Another significant input of this study was the generation of many digitally reconstructed skulls that were uploaded to Digimorph (http://digimorph.org/index.phtml). It later benefited several studies on snake evolution, including this dissertation.

The description of the snake fossil *Kataria anisodonta*, and its phylogenetic analyses, found it to be nested within crown snakes, same for Madtsoiidae and Pachyophiidae (Scanferla et al., 2013). *Najash* and *Dinilysia* were found as representatives of the sister lineages of crown snakes. Instead of the early ancestor, *Kataria* offered information about the ecological radiation of "macrostomata", demonstrating that considerable changes in the maxillary bone were already underway in the early Tertiary.

These studies were followed by a series of redescriptions and phylogenetic reanalysis of fossil snakes that were meant to address differential anatomical descriptions. The reexamination of the type and referred materials of *Najash* found that marine fossil snakes were "the most basal" even though results were uncertain as two other equally supported phylogenies had soft polytomies (Palci et al., 2013a). These authors criticized the use of disarticulated elements to describe fossil species and interpreted that the long body length of *Najash* would preclude it to have been a fossorial (see Albino, 2011). A redescription of *Pachyrhachis*, *Haasiophis*, and *Eupodophis* found them to be either the sister group of all other snakes or nested inside crown snakes (Palci et al., 2013b). It is interesting to notice that uncertainty in phylogenies was acknowledged in these studies and alternative results from the same analyses were openly described. The redescription of *Dinilysia* led to the claim of the absence of a Crista Circumfenestralis in that fossil - a trait commonly associated with snake morphology (Palci & Caldwell, 2014).

It becomes clear that the problem of the ecological origin of snakes was becoming ever more intractable based on qualitative morphology and phylogenetic analyses of fossils together with extant species. Not surprisingly, new approaches to tackle the problem of the origin of snakes began to appear like ontogeny, ancestral state estimation, and geometric

morphometrics.

The ontogenetic description of *Dinilysia* fossils was a step forward from the pure qualitative descriptions of adult specimens and their phylogenetic analyses. The investigation of a few post-natal specimens led to the suggestion that snakes developed faster (Scanferla & Bhullar, 2014).

Another innovative approach to the field, the statistical estimation of snake ancestors, which was inferred from multiple methods of ancestral-state estimation, indicated that early ancestors were nocturna and did not have capacity to constrict (Hsiang et al., 2015). They likely ingested vertebrate and invertebrate prey with a soft body and were terrestrial (although they might have also exhibited semi-fossorial behavior but were very unlikely aquatic/marine). It was estimated to have inhabited warm, well-watered, and well-vegetated environments. In terms of the phylogeny, they found that *Coniophis*, *Najash*, and *Dinilysia* were the sister species of all the other snakes (including Madtsoiidae and Simoliophiidae, both nested inside Alethinophidia). Previously, studies deduced the ecology of snakes based on the sister-group position of fossil taxa or extant species, a particularly problematic approach (see discussion).

Another innovative approach was devised by Yi and Norell (2015) who analyzed the inner-ear shape of extant snakes and *Dinilysia*. They found an association between its shape and habitat. They used that correlation to estimate the paleoecology of *Dinilysia*, which was estimated to be a fossorial species, despite its large body size. They pointed out that this finding supported a fossorial origin for crown snakes. However, that conclusion was later disputed based on more samples, but no clear scenario was favored in this last case: semiaquatic or semi-fossorial (Palci et al., 2017).

In the tradition of increasing the number of samples in phylogenies, a large-scale combined phylogenetic study found that all included fossils were nested within crown snakes while mosasaurs were the sister-group of snakes (Pythonomorpha) (Reeder et al., 2015). The ecology of snake ancestors was not discussed, but instead the importance of large-scale combined analyses.

The redescription and reinterpretations of fragments are now attributed to Jurassic snakes (*Parviraptor estesi, Diablophis gilmorei, Eophis underwoodi,* and *Portugalophis lignites*), and their phylogenetic analysis, retrieved all of them as the sister species of all the other snakes (Caldwell et al., 2015). This led to the suggestion that snakes had undergone an early ecological and morphological radiation in insular as well as continental areas. Furthermore, the snake ancestor was hypothesized as being altogether marine and terrestrial, perhaps implying a semi-aquatic or semi-terrestrial ecology.

Another controversial discovery was the description of the four-legged fossil *Tetrapodophis amplectus*, whose body structure and size were interpreted as a snake adapted to a fossorial behavior (Martill et al., 2015).

In a phylogeny, it was recovered to be the sister species of all the other snakes. The Jurrassic fossils were not added. They suggested a fossorial origin of snakes.

Another innovative approach to the field of early snake evolution was the use of linear morphometrics analysis to investigate the body ratios of *Tetrapodophis amplectus* in comparison to fossorial snakes and lizards of different ecologies (Lee et al., 2016). Their quantitative results supported a fossorial ecology for this fossil, compatible with its initial ecological description (Martill et al., 2015). Surprisingly, Lee et al. (2016) concluded that a fossorial over a marine hypothesis could not be favored. Their discussion added extra confusion around a fossil that was under scrutiny by the scientific community due to legal concerns and disputes about its taxonomy, but no publications in peer-reviewed journals have yet challenged conclusions by Martill et al. (2015).

To unite molecular and morphological data in phylogenies, Pyron (2017) analyzed several snake fossils species in a combined analysis that was calibrated by fossil dates and found that all analyzed snake fossils were nested inside the crown-snake clade or then that *Najash* and *Dinilysia* were the only stem snakes. On the other hand, in a combined phylogenetic tree, Harrington and Reeder (2017) found that Jurrasic snake fossils, *Najash,* and *Coniophis* were the sister lineages of all the other snakes. They also performed ancestral state estimation and found that the earliest snake ancestors had a limited gape, whereas the alethinophidian ancestor was a macrostomata, and that multiple independent losses of this feature were linked with fossoriality in snakes.

A description of a new lizard fossil *Megachirella wachtleri* led to a new phylogenetic hypothesis based on morphology in which *Najash* and *Pachyrhachis* were grouped, and with *Dinilysi*a, were the sister species of all the other snakes (Simões et al., 2018). The authors claimed that, for the first time, morphological and molecular data agreed regarding the early squamate evolution.

More recently, Garberoglio et al. (2019a) analyzed new fossils findings attributed the snake *Najash*. The estimated phylogeny is unique so far in recovering a sister clade to crown snakes composed of fossils with different ecologies. Other researchers will need to investigate those new findings independently. As shown above, interpretations of the same fossils differ significantly among paleontologists and phylogeneticists. For instance, Garberoglio et al. (2009b) provided very different phylogenies with nested simoliophiids among crown snakes instead. They do not discuss the ecological origin of snakes but for the interpretation that it likely had a wide-gape size.

Finally, heterochrony - changes in the rate and onset-offset of developmental processes - had been also associated with the origin of the snake body plan. Irish (1989) hypothesized that the ophidian skull evolved by local and global heterochronic patterns. The first formal attempt to

investigate the role of heterochrony concerning the early ecology of snakes was inconclusive, but the analysis of ossification sequences pointed out for local heterochronic patterns, for example, in the snout of snakes, linked with dissociations of bone sutures and enhanced skull flexibility (Werneburg & Sánchez-Villagra, 2015). This feature is thought to have contributed to the radiation of snakes (Irish, 1989; Werneburg & Sánchez-Villagra, 2015).

In sum, phylogenetic methods and interpretations of the same fossils can lead to different results and conclusions. It is also clear that the analysis of morphology combined with phylogenies would not be enough to detangle the ecological origin of snakes. Rieppel and Kearney (2001) wrote that "The origin of snakes has recently become a palaeontological 'hot potato'" (p. 110). Morphology, paleontology, and phylogenetic analysis are relevant, but hold three major caveats that have been the source of many ambiguities: I) sampling, II) methodological, and III) conceptual.

1.3.2) MAJOR CAVEAT (I): SAMPLING

Snake skulls are less represented in the fossil record than vertebral remains (e.g., Rage & Werner, 1999; Rage & Escuillié, 2003; Caldwell et al., 2015). For example, *Simoliophis (marine)*, *Lapparentophis (terrestrial)*, *Coniophis (fossorial)*, *Seismophis, Lunaophis* (aquatic) and *Titanoboa* (semi-aquatic) are better known from vertebrae (e.g., Hoffstetter, 1960; Cuny et al., 1990; Vullo, 2019; Lee & Scanlon 2002a; Longrich et al., 2012; Head et al., 2009; Hsiou et al., 2014; Rage et al., 2016; Albino et al., 2016).

The earliest skull fragments attributed to snakes were dated to the Jurassic: *Parviraptor estesi, Diablophis gilmorei, Eophis underwoodi,* and *Portugalophis lignites* (Caldwell et al., 2015); all previously classified as lizards (Evans, 1994). Some authors consider them as putative snakes (Martill et al., 2015; Evans, 2015).

Fossilized skull fragments have been described for the Cretaceous (Cenomanian) marine *Pachyophis woodwardi* (Nopcsa, 1923; Rage, 1984; Lee et al., 1999b; Caldwell & Albino, 2001; Houssaye, 2010; Rieppel & Zaher, 2000); the Cretaceous (Cenomanian) marine *Mesophis nopcsai* (Bolkay, 1925); an unidentified Cretaceous snake (Rage & Werner, 1999), the Upper Cretaceous (Maastrichtian) fossorial snake *Menarana nosymena* (Laduke et al., 2010), the Cretaceous (Maastrichtian) fossorial *Coniophis precedens* (Longrich et al., 2012, Palci et al., 2013b), the Palaeocene (Danian) *Kataria anisodonta* (Scanferla et al., 2013) (Figure 3A1, 3A2), the Oligocene *Rottophis atavus* (Szyndlar & Böhme, 1996), the Eocene *Ogmophis compactus* (Smith, 2013), Eocene Booids (McCartney &Seiffert, 2015), snakes from different families spamming from the Cretaceous to the Paleogene (Scanlon, 1996; Scanlon & Lee, 2002; Scanlon, 2003), and the Miocene snakes *Nanowana godthelpi and N. schrenki (*Scanlon 1997).

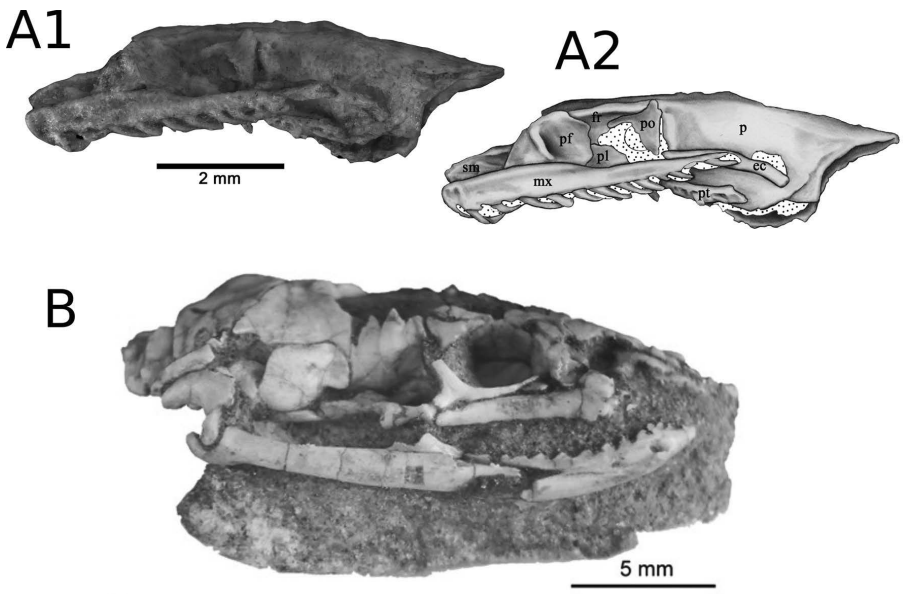

Figure 3 The skulls of two snake fossils. A1: photograph of the skull of *Kataria anisodonta* (MHNC 13323) and A2: half-tone drawings in left lateral view. Source: Scanferla et al., (2013). License: open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. B: *Najash*, a skull with atlas-axis in right lateral view (MPCA 500). Source: Garberoglio et al. (2019). License: open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial license, which permits use, distribution, and reproduction in any medium, so long as the resultant use is not for commercial advantage and provided the original work is properly cited.

Discoveries or redescriptions of articulated skulls with different levels of taphonomic issues and missing elements were also reported: the Cretaceous fossorial *Tetrapodophis amplectus* (Martill et al., 2015); the Cretaceous (Cenomanian) marine *Eupodophis descouensi (*Rage & Escuillié, 2000; Rage & Escuillié, 2002; Rieppel & Head, 2004; Houssaye et al., 2011; Palci et al., 2013b*);* the Cretaceous (Maastrichtian) terrestrial *Sanajeh indicus* (Wilson et al., 2010); the Eocene minute boas *Messelophis variatus, Rieppelophis variatus,* and *R. ermannorum* (Baszio, 2004; Schaal & Baszio, 2004; Scanferla et al., 2016)*;* the Eocene terrestrial *Eoconstrictor fischeri* (Smith & Scanferla, 2016; Scanferla & Smith, 2020); the Eocene aquatic (perhaps terrestrial, see Massalongo, 1859) *Archaeophis proavus* (Massalongo, 1859; Janensch, 1904; Janensch, 1906; McDowell & Bogert, 1954) and *A. turkmenicus* (Tatarinov, 1963; Tatarinov, 1988).

A few species with articulated elements, yet with differential levels of taphonomic disturbances, have described and reconstructed skulls:

a) *Pachyrhachis problematicus* was re-described as a marine simoliophiid snake and dated from the Cretaceous (Cenomanian) of Israel. A complete, yet crushed skull was preserved (Caldwell & Lee 1997; Caldwell & Albino 2001). Originally, this fossil was classified as a lizard (Haas, 1979, 1980a,b; Wallach, 1984). Its skull has been reconstructed in lateral view (Scanlon, 1996; Caldwell & Lee 1997; Polcyn et al., 2005; Palci et al., 2013b).

b) *Haasiophis terrasanctus* is a marine simoliophiid snake described from the Cretaceous (Cenomanian) of Israel and with preserved skull elements but also a crushed skull (Tchernov et al., 2000; Rieppel et al., 2003). Its skull was reconstructed in lateral view (Conrad, 2008).

c) *Dinilysia patagonica* is a fossil snake described from the Cretaceous (Santonian-Campanian) of Patagonia (Argentina) that was described from articulated and disarticulated skull parts (Smith-Woodward, 1901; Estes et al., 1970; Frazzetta, 1970). New specimens have been described and compared (Caldwell & Albino, 2002; Caldwell & Calvo, 2008; Zaher & Scanferla, 2012; Palci & Caldwell, 2014; Albino, 2011; Albino & Brizuela, 2014; Triviño et al., 2018), including ontogeny (Scanferla & Bhullar, 2014) and geometric shape of the inner ear (Yi & Norell, 2015; Palci et al., 2017). The paleoecology of this fossil is debatable: terrestrial (Caldwell & Albino, 2001), fossorial (Zaher & Scanferla 2012; Yi & Norell, 2015), aquatic (Frazzetta, 1970) or semi-aquatic (Palci et al., 2017). Its skull was reconstructed in lateral view (Zaher & Scanferla, 2012).

d) *Najash rionegrina* (Figure 3B) is *a* Cretaceous (Cenomanian–Turonian) snake from Patagonia (Argentina) and had been previously described based on skull fragments (e.g., braincase) (Apesteguia & Zaher, 2006; Zaher et al., 2009; Palci et al., 2013a) and more recently by a nearly complete and well-preserved skull in three-dimensions (Garberoglio et al., 2019a,b). This snake is interpreted as being terrestrial: surface dwellers and/or fossorial (Apesteguia & Zaher 2006; Zaher et al., 2009; Palci et al., 2013a; Garberoglio et al., 2019a,b). The shape of the skull is well-preserved, not requiring major reconstructions. Sadly, it could not be analyzed in time to be included in this dissertation.

e) *Wonambi sp.* (Figure 4B) is a madtsoiid fossil described from the Pliocene-Pleistocene (*W. naracoortensis*) and the Oligocene-Miocene (*W. barriei)* of Australia that, have preserved disarticulated skull elements (Barrie, 1990; Scanlon & Lee, 2000; Scanlon, 2003; Scanlon, 2005). Only about half the skull is available from the best-known species of *W. naracoortensis* (Scanlon & Lee, 2000; Scanlon, 2005). The ecology of this snake has been debated as climbing

(Barrie, 1990), semi-aquatic or terrestrial (Scanlon & Lee, 2000), or unresolved (maybe generalist?) (Palci et al., 2018). Its skull was reconstructed in lateral view and is a chimera of *W. naracoortensis and W. barriei* (Scanlon & Lee, 2000) or lacks important skull parts (*W. naracoortensis)* (Palci et al., 2018).

f) *Yurlunggur sp*. (Figure 4A) is an Oligocene-Miocene madtsoiid snake from Australia that has been described from two partial skeletons which together represent almost the complete skull (like lacking a supratemporal) (Scanlon, 2003; Scanlon, 2006). Paleoecological inferences based on its inner-ear shape found it to be of uncertain habitat ecology, perhaps semi-aquatic and/or semi-fossorial (Palci et al., 2018). The original fossil is partially disarticulated, and its skull was reconstructed in lateral view (Scanlon, 2006; Palci et al., 2018).

Figure 4 Skull reconstruction of snake fossils. Digital renderings of the reconstructed skulls (based on known elements) of (A) *Yurlunggur* sp. (QMF45391, QMF45111) and (B) *Wonambi* naracoortensis (SAMA P30178A, SAM P27777). Source: Palci et al. (2018). License: published by the Royal Society under the terms of the Creative Commons Attribution License - unrestricted use, credit author, and source.

Conrad (2008) stated that incomplete taxon sampling, including fossils, is likely a major contributing factor to the absence of a consensus

about squamate interrelationships. On the other hand, Simões et al. (2017) defend that more than an increasing number of taxa and characters, special attention should be given to the definition of characters and homology. Thus, methodological approaches play a relevant role as introduced below.

1.3.3) MAJOR CAVEAT (II): METHODOLOGICAL

The evolutionary relationships of snakes and lizards have been extensively investigated by molecular (Forstner et al., 1995; Heise et al., 1995; Macey & Verma, 1997; Saint et al., 1998; Hedges & Poling, 1999; Rest et al., 2003; Vidal & Hedges, 2004, 2005, 2009; Fry et al., 2006; Kumazawa, 2007; Albert et al., 2009; Castoe et al., 2009; Voronov et al., 2011; Mulcahy et al., 2012; Wiens et al., 2012; Pyron et al., 2013; Zheng & Wiens, 2016; Irisarri et al., 2017; Streicher & Wiens, 2017), morphological (Estes et al., 1988; Caldwell & Lee, 1997; Lee, 1997, 1998, 2000, 2005a,b; Zaher, 1998; Lee & Caldwell, 1998; Lee et al., 1999a,b; Zaher & Rieppel, 1999; Caldwell, 1999; Scanlon et al., 1999; Lee & Caldwell, 2000; Tchernov et al., 2000; Scanlon & Lee, 2000; Rage & Escuillié, 2000; Caldwell, 2000; Lee & Scanlon, 2002a,b; Zaher & Rieppel, 2002; Rieppel et al., 2002; Rage & Escuillié, 2003; Scanlon, 2006; Apesteguía & Zaher, 2006; Conrad, 2008; Zaher et al., 2009; Wilson et al., 2010; Longrich et al., 2012; Zaher & Scanferla, 2012; Gauthier et al., 2012; Scanferla et al., 2013; Palci et al., 2013a,b; Martill et al., 2015; Caldwell et al., 2015; Simões et al., 2017; Skawiński & Borczyk, 2017; Simões et al., 2018; Garberoglio et al., 2019a,b) and combined inferences (Lee et al., 2007; Lee, 2009; Wiens et al., 2010; Jones et al., 2013; Hsiang et al., 2015; Reeder et al., 2015; McMahan et al., 2015; Harrington et al., 2016; Pyron, 2017; Harrington & Reeder, 2017; Koch & Gauthier, 2018; Garberoglio et al., 2019a; Burbrink et al., 2020). Despite this large effort that began in the 1980s, phylogenies continue contradicting each other in significant ways, particularly between morphology versus molecular inferences, and the position of fossils in combined analyses.

In an overview, attempts to resolve the affinities of snakes within squamates have suffered from: choice of the type of data and analysis; variation in the interpretation of anatomical features, which affects their coding - perhaps the most contentious aspect for morphological data; and discordances between morphologists, paleontologist and phylogeneticists regarding strategies to cope with convergence (homoplasy). This is an issue particularly relevant for fossils which has been known to affect dramatically the phylogeny of snakes and lizards as introduced for the history of the debate of the ecological origin of snakes.

Giant taxon-character matrices have become more common for the generation of phylogenetic hypotheses for squamates. Simões et al. (2017) discussed in detail that problematic definition of characters can reduce the capacity of phylogenetic analyses to generate well-supported clades. Those

authors observed that when characters from other matrices (Conrad, 2008; Gauthier et al., 2012) are reinterpreted or deleted, as expected, phylogenetic reconstructions were impacted.

Simões et al. (2017) also reviewed problematic definitions of characters and character-states, fitting them into two types: Type I (discrete) and Type II (continuous). Some of the problematic characters would include conjunction (two or more structures are present in the same individual and are considered homologous even though they are not serial), continuous data unjustifiably treated as discrete, biogeographic characters, characters with vague explanations, taphonomy-biased characters, among others. Others can be considered subjective like problems with the interpretation of the morphology during character construction.

Differential use of phylogenetic data, methods, and approaches to analyze phylogenetic informative data can influence tree topologies (Losos et al., 2012; Lee & Palci, 2015; Simões et al., 2017). That is not limited to morphological characters. Differences can be substantial between phylogenies inferred based on nuclear genes, mitochondrial genes, and morphology (e.g., Lee, 2009). For example, for molecular studies, two common approaches for estimating phylogenies in species-rich groups are to sample many loci for few species (e.g., phylogenomic approach) (e.g., Wiens et al., 2012), or sample many species for fewer loci (e.g., supermatrix approach) (Pyron et al., 2013). These approaches have been combined and found that missing data did have a great impact in the topology of higher-level relationships and the tree statistically resembled more that of the first approach (Zheng & Wiens, 2016).

Combined phylogenetic inferences are becoming the scientific norm. Wiens et al. (2010) showed that combined data can alter the location of extinct and extant species in the topology and so produce a truly combined phylogenetic hypothesis. However, differences in the placement of fossils are significant between studies to the extent that downstream comparative analyses require taking into consideration alternative hypotheses. Moreover, it is common that rogue taxa are fossils, which reduce overall tree support (Reeder et al., 2015; Pyron, 2017). In this regard, Pyron (2017) assertively discussed that different morphological data sets produced by different authors do contain both different characters and different states for the same or similar characters, resulting in dramatically different placements for many important fossil lineages. This agrees with Simões et al. (2017) and Lee and Palci (2015). The author urged for extra efforts to standardize ontology for morphology that is expected to resolve incongruences in a robust phylogeny.

Additionally, Zaher et al. (2009) discussed the impact on phylogenies of a priori evolutionary expectations for coding of limb versus skull anatomies and setting what is to be considered “primitive” or “derived” features, which can lead to major phylogenetic disagreements. For instance, there is an ongoing debate if the macrostomate skull or limbs

have reappeared or been lost several times (e.g., Coates & Ruta, 2000; Rieppel et al., 2003). This debate was configured into the head-first (Caldwell et al., 2015) and body-first hypotheses (Longrich et al., 2012). Thus, interpretation of anatomy can be extremely variable between anatomists, paleontologists, and morphologists.

Lee (2000) wrote that "the caveat must be made that any separation of characters into distinct 'datasets' for analysis might be subjective and artificial" (p. 102). That conclusion is also backed by Rieppel and Kearney (2002) and Rieppel et al. (2003), who equally stated that “Because the assessment of morphological characters necessarily entails a conceptual element of abstraction, there is also a threat that preconceived notions of phylogeny influence character analysis.” (p. 59). The problem of interpretation of the homology of skull bones can be also exemplified by the uncertainty of the presence or absence of a jugal and crista circumfenestralis in fossil snakes (Caldwell & Lee, 1997; Zaher & Rieppel, 1999; Tchernov et al., 2000; Caldwell & Albino, 2002; Polcyn et al., 2005; Caldwell, 2007; Zaher & Scanferla, 2012; Palci & Caldwell, 2014).

Finally, convergence offers core methodological issues (Lee, 1998), and a detailed definition and contextualization of this biological phenomena are shown in the section covering geometric morphometrics. As of general note, Gauthier et al. (2012) stated that "Longbodied, limb-reduced, "snake-like" fossorial lizards—most notably dibamids, amphisbaenians, and snakes—have been and continue to be the chief source of character conflict in squamate morphological phylogenetics" (p. 3). Although morphology seems more prone to convergence, molecular data can also be subject to it (Castoe et al., 2014). Thus, homoplasy has been pointed out to overcome the phylogenetic signal and lead to spurious groups (Wake et al., 2011; Wiens et al., 2006).

All in all, relying only on fossils and phylogenies to investigate the ecological origin and radiation of snakes is not enough. It should then be combined with other approaches with a clear conceptual framework. However, as introduced below, the field of snake evolution suffers from evolutionary misconceptions that goes beyond the definition of characters, character-states, and homology.

1.3.4) MAJOR CAVEAT (III): CONCEPTUAL

The two previous caveats are intrinsic to any study, although some are products of subjectivity. There are approaches to circumvent or minimize them, which are gradually being considered to produce phylogenetic trees and the description of morphological features in larger datasets. However, a core conceptual issue influences research design, expectations, hypotheses, interpretation of results, focus of discussions, and conclusions. Conceptual issues are hard to be tackled, as presented in section 1.1., and mostly uncomfortable to be pointed out (see discussion).

A major conceptual issue is that most ecological inferences of snake ancestors were made by simply implying that a sister-group taxon position in cladograms and phylogenetic trees, particularly of fossils, is enough to grant that tit represents the same or very similar ancestral ecomorphology for all the other crown or ingroup snakes. The most representative exceptions to this conceptual pattern are Werneburg and Sánchez-Villagra (2015), Hsiang et al. (2015), Yi and Norell (2015), and Harrington and Reeder (2017), which all have employed ancestral state estimations instead of being limited to assumptions associated with a hierarchical rationale linked with assumptions of phenotypic evolution. Sadly, these studies did not provide enough resolution to distinguish between the posited ecological hypotheses for the origin of snakes (Hsiang et al., 2015; Werneburg & Sánchez-Villagra, 2015), but they strongly rejected a marine origin for snakes (Yi & Norell 2015; Hsiang et al., 2015). Of note, ancestral-state estimation is not to be taken as the sole evidence, more details to be cautious about this methodology is shown in the discussion and contextualized to the present research and methodological issues such as "the sister-group fallacy".

I hope to convince you in my dissertation that the mentioned conceptual issues have been extremely resistant to conceptual change. They are usually connected to misleading notions of phenotypic evolution, incorrect notions of primitive versus derived lineages or organisms, and implicit notions of "missing links" or perfect intermediate forms. These issues are found within peer-reviewed scientific publications, detailed in the discussion, and is particularly explicit in the scientific news. The following examples illustrate my point: *(i)* ""It could very well be that what you would see in terms of the missing link features is that this animal would have had four legs and a short body," Caldwell said." (Casey, 2015); *(ii)* "These primitive snakes with little legs weren't just a transient evolutionary stage on the way to something better," Professor Lee said." (StreetWise Media, 2019).

So, an integrative approach with a strong conceptual framework was needed previous to this research program. In this regard, the analysis of the skull anatomy using geometric morphometric approaches and that associated with modern comparative analyses seemed to have the potential to shed light on the early ecology of snakes based on other studies in different groups (Klingenberg, 2010). In support of the relevance of anatomical shape to understand snake evolution, the comparison of the inner-ear shape of snakes showed a partial correlation between it and habitat ecology (Yi & Norell, 2015; Palci et al., 2017). Additionally, recent geometric morphometric studies have combined different sources of data and generated insights about skull evolution in several lizards and snake lineages, but never for both groups together, neither the ancestral ecomorphologies of snakes (e.g., Stayton, 2005; Sanger et al., 2013; Hipsley & Müller, 2017; Esquerré & Keogh, 2016; Fabre et al., 2016; Klaczko et al.,

2016). Thus, these studies provided clues that an integrative approach using geometric morphometrics could be a promising alternative.

1.4) GEOMETRIC MORPHOMETRICS

Geometric morphometrics is an approach that is used to quantify shape and size, which together make up biological "endless forms of most beautiful". In the next section, I reviewed the concept of form-shape, morphometrics, and their applications in the study of skull evolution and development in squamates. This approach was proved valuable for the study of snake origin.

Kendall (1977) was a pioneer in the study of shape, and he stated that "if we are not interested in the location, orientation or scale of the resulting configuration, then we find ourselves working with a continuous stochastic process describing its change of shape." (p. 428). Thus, in geometric morphometrics, a shape can be summarized as all the geometric information that remains when location, scale, and rotational effects are filtered out from a biological structure (Zelditch et al., 2004). The minimum geometric unit of shape analysis in geometric morphometrics is a triangle of landmarks but biological structures tend to be more complex (Bookstein, 1989).

In landmark-based geometric morphometrics, the shape is a feature of the whole configuration of landmarks (Zelditch et al., 2004). Landmarks are a set of coordinates that are comparable between biological structures (Zelditch et al., 2004). In biology, landmarks are discrete anatomical loci that can be identified across species through homology (Zelditch et al., 2004). Homology is likeliness because of common descent, so ancestry, with a hierarchical structure departing from genes to the phenotype (Hall, 2013). Regarding skeletal structures, homology is ascertained commonly by bone topology and sutures between bones. Landmarks can describe shapes in two-dimensions (2D) and three-dimensions (3D) by cartesian coordinates (Zelditch et al., 2004). 2D landmarks (x,y) are placed on pictures (dorsal, ventral, or lateral view) (Figure 5).

Precision is increased by exploring 3D landmarks (x,y,z) in digitally reconstructed structures. The Procrustes Generalized Least-Square method became the norm for the superimposition of landmark configurations (Rohlf & Slice, 1990). It eliminates non-shape variation (Figure 5). Out of feeding curiosity, the name Procrustes is prevenient from Greek mythology (Zelditch et al., 2004). Procrustes used to fit his victims to a bed by stretching or compressing them, and so he minimized the difference between the bed itself and its victims (although he painfully interfered in their body shapes). In a nutshell, this mathematical treatment minimizes the differences between landmark configurations in comparison to a referential shape, as the algorithm iteratively overlaps configurations so that homologous landmarks match as closely as possible.

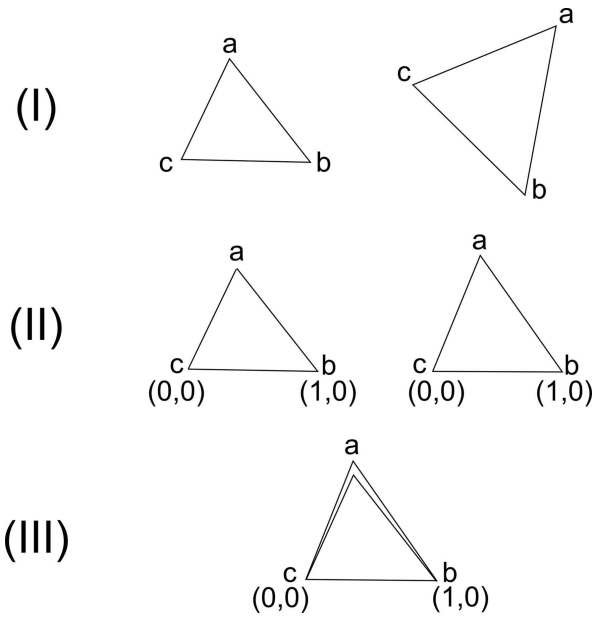

Figure 5 Procrustes superimposition of two triangles that represent two biological structures. Landmarks: a, b and c. (I) the two landmarked triangles; (II) the same two triangles after being translated, rotated, and rescaled; (III) superimposed shapes. (Modified from Zelditch et al., 2004).

1.4.1) A BRIEF HISTORY OF THE ANALYSIS OF FORM AND SHAPE

The concept of form is first formalized in the occidental tradition by Plato (c. 428–c. 348 BCE) (Oxnard et al., 2000) and later Aristotle (384–322 BCE), who studied at Plato's Academia. In *Timaeus* (360 BC), Plato compared the five elements (earth, air, water, fire, and plasma) that were understood to compose our imperfect reality with regular geometric forms (cube, octahedron, icosahedron, tetrahedron, and dodecahedron) (Lamb, 1925), some sort of form and function relationship. In the Socratic dialogues documented in *The Republic* (375 BC), Plato presents us with the allegory of the cave. Arguably, it is the most famous analogy in the history of philosophy, in which the process of being educated about forms is narrated (Shorey, 1969). Perceived forms are projections of the perfect ones outside of the cave or the world we are imprisoned in. This resonates strongly within the field of morphometrics. In *Metaphysics*, Aristotle narrated Plato's view on forms as the one perfect instance of what they referred to (Taylor, 1801), but, differently, chose to divide objects into form and matter.

Interestingly, parallels with the concepts of early Greek philosophers

can be drawn concerning current biology. First, the holotype of species is somehow analogous to Plato's form. Moreover, genus and species were coined by Aristotle himself in *The History of Animals* (4th century BC) (Thompson, 1910; Gill, 1911). Aristotle had indeed some things to say about the ecomorphologies of snakes and lizards. He wrote, "further, in respect to the locality of dwelling place, some creatures dwell underground, as the lizard and the snake; others live on the surface of the ground, as the horse and the dog." (Book I, part 1), and "the serpent genus is similar and in almost all respects furnished similarly to the saurians among land animals, if one could only imagine these saurians to be increased in length and to be devoid of legs" (Book II, part 17). Second, the association between geometric forms with functional outcomes is sound for biological shapes as they are often correlated with ecological functions (form-and-function relationship) (Russell, 1916; Thompson, 1917; Banavar et al., 2014; Ball, 2016), so common that an applied (engineering and technology) field was developed from this association: biomimetics (Benyus, 1997; Bhushan, 2009); alike influencing designers and artists (Verma & Punekar, 2019). Third, Plato's narrative that regular shapes did not transform equally into each other has parallels with the phenotypic evolution of complex traits (Alberch, 1980). These parallels are not to say that Plato and Aristotle had the current understanding of the relationship between shape and ecology, but that the geometric approach for comparative biology has a long history.

Comparative anatomy plays a fundamental role in the investigation of form and function. The early debate between the functional approach of Georges Cuvier, which held that function is the primary determinant of form, and the transcendental approach of Etienne Geoffroy Saint-Hilaire, which in turn posited that form determines function, is one that would become of major relevance within the framework of evolutionary biology (Cooke & Terhune, 2015). Comparative anatomy is then in the heart of evolutionary biology (Darwin, 1859), in which that dichotomic debate turned into of adaptationism (natural selection) versus structuralism (constraints) (Gould, 2002), as well as a combination of both in the context of evo-devo (Alberch, 1980; Salazar-Ciudad & Marín-Riera 2013). Yet, morphology and its association to function have been traditionally described by discrete traits and their linear metrics, limited for quantification of biological shapes (Cooke & Terhune, 2015).

The interest for populational data and quantitative traits began to grow between the 19th and 20th centuries, which created the conditions for the rise of statistics and traditional morphometrics (Oxnard et al., 2000). Morphometrics can be defined as the quantitative description, analysis, and interpretation of shape and shape variation in biology (Rohlf, 1990). Bookstein (1991) defined morphometrics as the statistical analysis of the covariance between shape and causal factors. Rohlf and Marcus (1993) redefined morphometrics as the study of biological shapes using multivariate analysis of object configurations. In other words, linear

morphometrics, or traditional morphometrics, deals with analyzing linear measurements of biological structures in a multivariate fashion to describe their shape (Claude et al., 2008). For example, systematic measurement of the cranium of humans and their statistical treatment became commonplace in biometrics, and, unfortunately, derailed towards racist ideologies such as eugenics and Nazism (Gould, 1996). Overcoming those biases, by the 1960s, analysis of complex biological shapes was benefited by the rise of computers and programming languages (Claude et al., 2008).

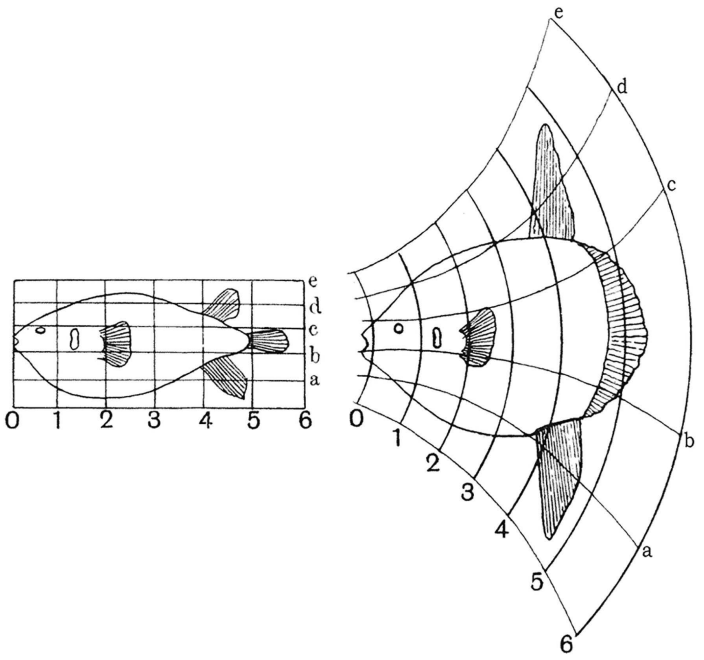

Figure 6 Image from "On Growth and Form". "Fig. 381 [left] is a common, typical *Diodon* or porcupine-fish, and in Fig. 382 [right] I have deformed its vertical co-ordinates into a system of concentric circles, and its horizontal co-ordinates into a system of curves which, approximately and provisionally, are made to resemble a system of hyperbolas. The old outline, transferred in its integrity to the new network, appears as a manifest representation of the closely allied, but very different looking, sunfish, *Orthagoriscus mola*. This is a particularly instructive case of deformation or transformation." (Thompson, 1917, pp. 751-752). License: Public Domain, available at https://www.gutenberg.org/files/55264/55264-h/55264-h.htm.

In contemporary times, linear morphometrics has been successfully employed in a variety of evolutionary studies of lizards and snakes, for example, *Anolis* lizards (Losos et al., 2009) and greater Antillean boas (Reynolds et al., 2016). Currently, it is complemented by geometric morphometrics, which describes biological shapes with a clear definition of

size, provides better resolution of quantified shapes, and controls for non-shape variation (Zelditch et al., 2004).

Geometric morphometrics can be traced to the seminal book "On Growth and Form" by D'Arcy Thompson (1917), in which was applied the concept of "deformation grids" to describe morphological changes between species and of one species into another (Figure 6).

A lengthy and amusing series of descriptions of mathematical transformations of biological forms were compiled and culminated in his "theory of transformations". D'Arcy Thompson saw the form of an object as a "diagram of forces", stating that by stretching and distorting one focal form, one produces closely related forms. However, Thompson's approach, and as acknowledged by himself, had been anticipated in the field of visual arts by the pioneering work of the German artist Albrecht Dürer in the 16th century (Abzhanov, 2017). In biology, it was pioneering to map biological forms onto a mathematical grid to describe and predict forms by deformation, changing, as it would be later better acknowledged, our perceptions of morphological changes throughout development and evolution (Arthur, 2006). His approach was formalized by geometric morphometrics only in the late '80s and since then it has become widely used (Bookstein, 1989). A scientific revolution took place in the quantification of morphological variation due to Geometric Morphometrics (Bookstein, 1998; Rohlf & Marcus, 1993; Adams et al., 2004; Klingenberg, 2010; Adams, 2013; MacLeod, 2017).

In 1993, Rohlf and Marcus proclaimed a scientific revolution:

> We are now in the midst of a revolution in morphometric methodology. The new approaches are more effective in capturing information about the shape of an organism and result in more powerful statistical procedures for testing for differences in shape. (p. 129).

In 2004, Adams and collaborators reinforced it in the publication “Geometric morphometrics: ten years of progress following the ‘revolution’:

> In the 1980s, a fundamental change began in the nature of the data gathered and analyzed. This change focused on the coordinates of landmarks and the geometric information about their relative positions. As a by-product of such an approach, results of multivariate analyses could be visualized as configurations of landmarks back in the original space of the organism rather than only as statistical scatter plots. (p. 5).

Ten years later, Adams and collaborators (2013) restated it in “A field comes of age: geometric morphometrics in the 21st century”:

> Twenty years ago, Rohlf and Marcus proclaimed that a “revolution in morphometrics” was underway, where classic analyses based on sets of

linear distances were being supplanted by geometric approaches making use of the coordinates of anatomical landmarks. Since that time the field of geometric morphometrics has matured into a rich and cohesive discipline for the study of shape variation and covariation. (p. 7).

1.4.2) A REVIEW: GEOMETRIC SKULL SHAPE STUDIES OF SQUAMATES

The last review on geometric morphometrics applied to the study of lizards and snakes was undertaken nearly a decade ago (Kaliontzopoulou, 2011). I largely updated that review in the following section. I subdivided it into four sections: morphology, ecology, phylogeny, and development. They correspond to sections in material/methods and results.

1.4.2.1) MORPHOLOGY

Morphology is an essential component of the genotype-phenotype map (Alberch, 1991). It can be described by multivariate morphospaces derived from morphometrics (Mitteroecker & Huttegger, 2009). Comparisons of morphospaces between studies are limited by differences in sample size, landmarks, and used methods, so their individual description bears limited interest for the scope of this dissertation. The following studies have reported some type of morphospace for lizards: Bruner and Costantini (2009), Daza et al. (2009), Huyghe et al. (2009), Leaché et al. (2009), Bruner et al. (2005), Harmon et al. (2005), Lamborot et al. (2005), Stayton (2005), Stayton (2006), Herrel et al. (2007), Kaliontzopoulou et al. (2007), Costantini et al. (2010), Raia et al. (2010), Piras et al. (2011), Zuffi et al. (2011), Kikukawa and Hikida (2012), Sanger et al. (2012), Urošević et al. (2013), Fabre et al. (2014), Openshaw and Keogh (2014), Sagonas et al. (2014), Urošević et al. (2014), McCurry et al. (2015), Hipsley et al. (2016), Tayhan et al. (2016), Dollion et al. (2017), Hipsley and Müller (2017), Openshaw et al. (2017), Taylor et al. (2017), Aguilar-Puntriano et al. (2018), Gabelaia et al. (2018), Hohl et al. (2018), Imhoff et al. (2018), Kazi and Hipsley (2018), Chaplin et al. (2019), Gray et al. (2019a, 2019b), and Urošević et al. (2019); and snakes: Manier (2004), Gentilli et al. (2009), Polachowski and Werneburg (2013), Ruane (2015), Davis et al. (2016), Esquerré and Keogh (2016), Fabre et al. (2016), Klaczko et al. (2016), Murta-Fonseca and Fernandes (2016), Silva et al. (2017), Esquerré et al. (2017), dos Santos et al. (2017), Silva et al. (2018), Tamagnini et al. (2018), Moshtaghie et al. (2018), Keates et al. (2019), and Sherratt et al. (2019b). Palci et al. 2016 was the only study that generated a morphospace combining snakes and lizards but included a very limited number of species that ultimately hampered the major conclusions that were achieved here with a larger sample size. Moreover, integrative studies between families are the most informative for macroevolutionary discussions and relevant

for this dissertation. Regarding that, Stayton (2005, 2006) generated the first large-scale morphospaces for lizards, finding strong phylogenetic structure and evidence of convergence. For snakes, Esquerré and Keogh (2016) compared several families of pythons and boas, which often overlap in the morphospace that generates ecomorphological patterns.

Allometry is another aspect that is relevant when investigating morphological patterns. D'Arcy Thompson (1917) compiled the importance of changes in size for changes in shape in biology. Thompson dealt more with the mechanical and physical factors underlying shape transformations but stressed the importance of rates of growth. The relationship between size and shape was further elucidated by empirical, theoretical, and mathematical investigations compiled in the book "Problems of Relative Growth" (Huxley, 1932). The term allometry was later coined to define the linear relationship between shape and size or the correlation between dimensions of a given structure with the body dimensions (Huxley & Teissier, 1936). Gayon (2000), after reviewing the history of this concept, suggested that Huxley did not fully acknowledge previous researcher's contributions and downplayed the role of allometry in the modern synthesis of evolution. The types of allometry were later clarified and their relationship to natural selection was proposed - a non-adaptive source of evolutionary change that is the consequence of the adaptive increase in size (Gould, 1966). Three types of allometry were defined: static – quantified often between adults within a species; evolutionary – among species; and ontogenetic - among developmental stages within species (Gould, 1966; Cheverud, 1982; Klingenberg, 2016). Over time, it became clear that allometry is of major relevance in evolutionary and developmental biology (Gayon, 2000). In geometric morphometrics, allometry is the study of the relationship between the centroid size and geometric shape in a multivariate sense, and it is thought to be subject to natural selection (Klingenberg, 2010).

Most studies in Squamata have reported significant allometry, the exception are five studies with lizards: Dollion et al. (2017), Băncilă et al. (2010), Stayton (2006), Vidal et al. (2006), Lamborot et al. (2005); and one with snakes: Gentilli et al. (2009). It is not the scope here to describe the allometric variation for each study, which is also quite extensive and intricated, but, most importantly, because they are not directly comparable due to differences in sample size and landmark configurations. Their value is relative to each study and so bears limited relevance for our studies and discussion but to show the importance to address the correlation between shape and size in evolutionary studies of skull shape. Of general note, the following studies have quantified allometry for lizards: Chaplin et al. (2019), Gray et al. (2019a,b), Urošević et al. (2019), Hohl et al. (2018), Imhoff et al. (2018), Kazi and Hipsley (2018), Hipsley and Müller (2017), Lazić et al., (2017), Openshaw et al. (2017), Powell (2017), Taylor et al. (2017), Hipsley et al. (2016), Lazić et al. (2016), Tayhan et al. (2016), Lazić

et al. (2015), McCurry et al. (2015), Fabre et al. (2014), Openshaw and Keogh (2014), Urošević et al. (2014), Bütikofer et al. (2013), Sanger et al. (2013), Urošević et al. (2013), Kaliontzopoulou et al. (2012), Kikukawa and Hikida (2012), Sanger et al. (2012), Hollenshead (2011), Ljubisavljević et al. (2011), Piras et al. (2011), Zuffi et al. (2011), Costantini et al. (2010), Ljubisavljević et al. (2010), Bruner and Costantini (2009), Daza et al. (2009), Huyghe et al. (2009), Kaliontzopoulou et al. (2008), Costantini and Bruner (2007), Bruner et al. (2005), Harmon et al. (2005), Monteiro (1999), Monteiro and Abe (1997); snakes: Murta-Fonseca et al. (2019), Sherratt et al.(2019), Tamagnini et al. (2018), Andjelković et al. (2017), Silva et al. (2017), Esquerré et al. (2017), Davis et al. (2016), Esquerré and Keogh (2016), Murta-Fonseca and Fernandes (2016), Segall et al. (2016), Mangiacotti et al. (2014), Henao-Duque and Ceballos (2013), Polachowski and Werneburg (2013), Manier (2004); and them combined: Palci et al. (2016). On the other hand, in the context of ontogenetic allometry, which is detailed in section 1.4.2.4, the description of studies has more relevance to this dissertation.

Previous to the first publication included in this dissertation (Da Silva et al., 2018), a combined morphospace of the skull shapes of snakes and lizards with the inclusion of most of their families, had not been quantified, so their relative shape-space occupation was unknown. Also, was not understood the relative importance of allometry between snakes and lizards, or if it had relevance for the origin of snakes.

1.4.2.2) ECOLOGY

Ecology can be summarized as the cause and consequence of biological diversity as well as its distribution in the ecosystems (biotic and abiotic elements that interact with organisms and emerge from them) (Losos, 2009).

Adaptations are understood to be commonplace, meaning features that have been shaped by natural selection in an ecological context that maximizes function with an increase of fitness (Ridley, 2003; Losos, 2009). Yet, morphological features do not have to follow an adaptive explanation. Adaptationism should be seen with caution and as an alternative explanation. For example, features that enhance fitness now might not have been selected for their current role (exaptation) (Gould & Vrba, 1982), they can be neutral, and be a product of different types of constraints (Gould & Lewontin, 1979). Additionally, phylogenetic inertia can lead to similarities due to the potential limitation of a lineage to respond morphologically to an existent selective pressure (Blomberg & Garland, 2002). Comparative methods make use of phylogenetic adjusted data to help distinguish between patterns created by selection, drift, and development (Klingenberg et al., 2011; Revell, 2012; Monteiro, 2013).

Despite nowadays caution, the relationship between form (shape)

and function (ecology) has been extensively investigated and demonstrated (e.g., Thompson, 1917; Abzhanov, 2017; Moon et al., 2019). For example, the interaction between morphology and ecology can be seen in the light of the concept of evolutionary novelty (Moczek, 2008; Pigliucci, 2008; Erwin, 2015), meaning diagnostic features (Peterson and Müller 2013) and evolutionary processes (Hallgrímsson et al., 2012; Alberch et al., 1979) that largely distinguish taxonomic levels by new morphological patterns associated with new functions (Zimm, 2019). Novelties can foster ecological radiations, the rapid diversification from an ancestral population with descendants using variable ecological opportunities (Osborn, 1902; Harmon et al., 2003; Stroud & Losos, 2016). Adaptive radiations are thought to be commonly associated with the origin of clades (Simpson, 1944, 1953). However, directional evolutionary paths might be more common due to selection and constraints rather than rapid evolution followed by stasis (Harmon *et al.,* 2010). Radiations can also be fostered by ecological opportunities such as the origin of new resources (e.g., food items), vacant habitats (e.g., island), and the reduction of competitors (e.g., mass extinction) (Simpson, 1953; Raup, 1994; Schluter, 2000). Interestingly, a recent study using machine learning to analyze a large database of fossils did not find clear correspondence between mass extinction and subsequent radiation, raising further insights that radiation is linked with key innovations (Cuthill et al., 2020). Finally, radiation in one group can also prompt radiation in another ("ecosystem engineering") (Losos, 2010) or then extinction (destructive creation) (Cuthill et al., 2020).

The environment is one of the major drivers of morphological evolution and is often paralleled with natural selection (Darwin, 1859; Ridley, 2003; Barton et al., 2007). In agreement, geometric morphometrics studies of lizards and snakes showed that skull shape is often found correlated with ecologies. Considering the association between skull shape and habitat for lizards, *Anolis* species have undergone repeated skull shape and ecological radiations upon island colonization (Harmon et al., 2005). *Liolaemus tenuis* (Vidal et al., 2005) and *Liolaemus pictus* (Vidal et al., 2006) have different skull shapes in the oceanic region (islands) in comparison to the mainland. Kaliontzopoulou et al. (2010) discovered that the head shape of *Podarcis* lizards varies substantially between a gradient of saxicolous to ground-dwelling habitats. Hollenshead (2011) compared *Egernia depressa*, *E. cygnitos*, and *E. epsisolus*, concluding that the lateral aspect of the cranium of the rock-inhabiting species differs from the log-inhabiting species (*E. depressa*). Sanger et al. (2012) found a partial association between the skull shape and ecomorphological categories of *Anolis* lizards in the Caribbean islands. Urošević et al. (2013) also detected a partial association between ecomorphologies and skull shapes for lacertid lizards. Openshaw & Keogh (2014) obtained that habitat use is predictive of shape disparity within phylogenetic lineages of varanids, especially for terrestrial forms. Sagonas et al. (2014) found that the head shape of Balkan

green lizard *Lacerta trilineata* differed between mainland and island populations. Hipsley & Müller (2017) found that the cranial shape of lacertid lizards varied significantly among most biome comparisons and semi-arid species were particularly distinct. Gray et al. (2019a) observed a significant association between cranial shapes and life habits for Australian agamid lizards in connection to adaptive radiation on a continental scale. Watanabe et al. (2019) retrieved a strong correlation between skull shape and habitat, including convergent fossoriality. Conversely, *Amphisbaena* might respond little regarding new environments in terms of cranial shape, being suggested that stabilizing selection maintains a conservative shape perhaps linked with conserved diets (Hipsley et al., 2016; Kazi & Hipsley, 2018). Kaliontzopoulou et al. (2018) found out that *Podarcis bocagei* and *P. vaucheri* head differences do not have a clear geographic, genetic, or environmental pattern. No association was also observed in the Moorish gecko *Tarentola mauritanica* (Massetti et al., 2019).

Considering the same form-and-function association for snakes, Fabre et al. (2016) found that homalopsid snakes that used burrows had different head shapes. Segall et al. (2016) also found that the aquatic environment drives the evolution of partially specialized head shapes. Esquerré and Keogh (2016) found that the skull of pythons and boas fit fairly within six ecomorphs.

The association between skull-head shape and habitat can also lead to a phenomenon known as morphological convergence (Stayton et al., 2006; Wiens et al., 2006; Losos, 2009; Losos, 2011). Convergence is one of the most important concepts in evolutionary biology and is understood to be ubiquitous in the history of life on this planet (Conway Morris, 2003). Darwin (1859) highlighted the importance of convergence as strong evidence for natural selection. Convergence has been identified even between multiple events of radiation (Mahler et al., 2013). It can occur also due to constraints (genetic, functional, or developmental) that bias the number of possible phenotypes (Gould & Lewontin, 1979; Maynard Smith et al., 1985; Brakefield, 2006) and evolutionary drift (Gould & Lewontin, 1979; Stayton, 2008). In geometric morphometrics, it can be identified when distantly related lineages overlap or are closely located in the (phylo)morphospace (Stayton, 2008; Wiens et al., 2006). Confirmation of convergent patterns requires the use of formal approaches, for example, distance-based metrics C1-C4 (Stayton, 2015).

Considering the convergence of shapes linked with habitats in lizards, *Anolis* species inhabiting similar vegetation stratus showed multiple convergence cases in their head shape (although individual traits are variably convergent) (Harmon et al., 2005), and partially in skull shapes (Sanger et al., 2012). Urošević et al., (2013) investigated lacertid lizards and observed that shrub-climbing species were clustered while saxicolous species were highly dispersed in the morphospace. Openshaw and Keogh (2014) found that there is convergence in head shape among rock-dwelling

species and arboreal species of Varanids. Hipsley and Müller (2017) observed that desserts can promote convergence in the skull shape of lacertid lizards. Similarly, skull convergence is seen in desert-dwelling species of *Liolaemus* with the distantly related *Ctenoblepharys adspersa* (Aguilar-Puntriano, 2018).

Considering the convergence of skull shapes to similar habitat for snakes, Esquerré and Keogh (2016) showed that pythons and boas display strong and widespread convergence of skull shapes in equivalent “niches” and that the history of phenotypic evolution strongly matches the history of ecological diversification; representing, as they pointed out, a strong case of parallel between ancient radiations and multiple cases of convergence.

Previous to the first publication included in this dissertation (Da Silva et al., 2018), it was unknown the level of association between skull shape and habitat for most lineages of lizards and snakes as well as between these two groups. Formal convergence metrics had not been applied before the quantification of potential skull shape convergences between those two groups. Finally, nobody had envisioned the use of skull shape to address the problem of the ecological origin of snakes.

1.4.2.3) PHYLOGENY

Phylogeny is a bifurcated representation of the historical relationships between biological organisms to their ancestors. As of curiosity, a diagram of a phylogenetic tree was the only image included in "On The Origin of Species" by Charles Darwin (1859).

A central concept in comparative studies is the phylogenetic signal (Blomberg & Garland, 2002; Revell et al., 2008), which is “the tendency for closely related species to display similar trait values due to their common ancestry” (Adams, 2014, p. 685). Detecting the phylogenetic signal is crucial for functional and ecological studies. Not surprising, as modification by inheritance is a pillar of evolution, so organisms are structured by their ancestral relationships (Darwin, 1859).

On the other hand, comparative analyses require independence among samples, and methods such as Independent Contrast (Felsenstein, 1985) and Phylogenetic Generalized Least Squares (PGLS) (Blomberg et al., 2012) have provided statistical power to adjust for inherent phylogenetic structure (Monteiro, 2013). In geometric morphometrics studies, two methods have been commonly employed to estimate phylogenetic signals. Klingenberg and Gidaszewski (2010) proposed fitting the phenotypic data to a phylogeny using algorithms of squared-change parsimony and estimating the sum of squared changes (SSC) of the trait across all branches of the phylogeny. Smaller values of SSC correspond to a better fit of the data to the phylogeny and thus represent a higher degree of phylogenetic signal. Adams (2014) developed a multivariate generalization of the *K* statistic of Blomberg et al. (2003) that can be applied to quantify phylogenetic signals

in multidimensional multivariate traits without ancestral estimation.

Comparative analyses also require a backbone phylogenetic hypothesis (phylogenetic tree). Evolutionary relationships among lizards and snakes are partially contentious but are expected to become more robust with larger molecular phylogenetic hypotheses (e.g., Pyron et al., 2013; Zheng & Wiens, 2016), revisited large-scale morphological data (e.g., Simões et al., 2017), and integration of large-scale molecular and morphological data (e.g., Burbrink et al., 2020; Garberoglio et al., 2019a; Reeder et al., 2015; Hsiang et al., 2015; Pyron, 2016; Harrington & Reeder, 2017; Koch & Gauthier, 2018; Simões et al., 2018; Zaher et al., 2019).

Well-supported branches in phylogenies are also important for the projection of the phylomorphospace (Sidlauskas, 2008). A plotted phylogenetic tree on the morphospace allows for the identification of patterns of shape evolution (e.g., evolutionary trajectory of shape change, convergence, and shape space occupation by different lineages) and estimation of ancestral shapes (Rohlf, 2002). Phylomorphospaces can also aid in the identification of areas of the morphospace that have adaptive attractors and in the identification of skull types prone to homoplasy (Hipsley & Müller, 2017). Ancestral-state estimation is commonly done by Square-Change Parsimony (Maddison, 1991). For instance, phylomorphospaces showed the direction of the evolutionary transitions of the skull and head shape in the radiation of monitor lizards (Openshaw & Keogh, 2014; Openshaw et al., 2017), convergence among lizards (Hipsley & Müller, 2017) and in snakes (Esquerré & Keogh, 2016; Esquerré et al., 2017; Fabre et al., 2016; Klaczko et al., 2016); and skull shape conservation in Caribbean amphisbaena lizards (Kazi & Hipsley, 2018). It aided in the identification of sexual dimorphism in tegu lizards (Fabre et al., 2014), casque-headed lizards (Taylor et al., 2017), and chameleons (Dollion et al., 2017).

Previous to the first publication included in this dissertation (Da Silva et al., 2018), an estimation of the phylogenetic signal for lizards and snakes together had not been performed. The integration of a phylomorphospace between lizards and snakes also remained undone. No formalized estimation of ancestral skull shapes had been performed. Skull shape disparity and evolutionary paths were unknown.

1.4.2.4) DEVELOPMENT

Development is the biological phenomena that culminate with the growth, differentiation, and maturation of multicellular organisms utilizing molecular mechanisms and cellular processes (Barresi & Gilbert, 2020). Evolution and development intersect in the research field of Evolutionary Developmental Biology (Gould, 1977; Goodwin, 1982; Raff, 1996; Hall, 1999; Carroll, 2005).

The rate of embryonic development in lizards and snakes is

temperature dependent (Köhler & Haecky, 2005), and is thought to have influenced the diversity of snakes in the Oligocene (Lynch et al., 2009). Furthermore, snakes and lizards keep growing after sexual maturity, making them excellent models for ontogenetic comparisons as aging is correlated with an increase in size (Piras et al., 2011). Additionally, the total length of the embryonic development of snakes and lizards has been extensively compiled (Köhler & Haecky, 2005).

Skull shape development can be investigated through ontogenetic allometry, which is commonly seen as a constraint to variation (directing the evolution of morphology) (Maynard Smith et al., 1985) or as a plastic trait that contributes to the rise of ecological specializations (Klingenberg, 2010). However, previous to the research included as part of this dissertation, only one study had applied geometric morphometrics to quantify the embryonic skull shapes of snakes and used it for producing a staging table for *Bothrops jararaca* (Polachowski & Werneburg, 2013). Instead, the postnatal development of the skull has been the major focus for lizards (Hipsley & Müller, 2017; Lazić et al., 2016; Lazić et al., 2017; Hipsley et al., 2016; Palci et al., 2016; McCurry et al., 2015; Urošević et al., 2013; Piras et al., 2011; Hollenshead, 2011; Raia et al., 2010; Monteiro & Abe, 1997) and snakes (Esquerré et al., 2017; Silva et al., 2018; Murta-Fonseca & Fernandes, 2016; Palci et al., 2016).

Among lizards, Monteiro and Abe (1997) identified shape changes throughout the post-natal ontogeny of *Tupinambis* that is paralleled by dietary shifts from carnivore to omnivore. Hollenshead (2011) identified skull shape differences of juveniles and adults of *Egernia cygnitos, E. epsisolus* with *E. depressa*, but skull views differed in their patterns for the angle of ontogenetic trajectories. Urošević et al. (2013) investigated the cranial shapes of twelve species of lacertid lizard, finding an increase in the morphological disparity throughout their ontogeny, and allometric trajectories of the shrub-climbing species were clustered while saxicolous species were highly dispersed. McCurry et al. (2015) included sub-adult specimens of *Varanus komodoensis and V. gouldii* that exhibited higher magnitudes of a strain than most other specimens, indicating that allometric differences between juveniles and adults may be of particular importance to trade-offs in cranial structural performance. Hipsley et al. (2016) found variation in skull shape to be allometric and conservative in the development of *Cynisca leucura* (Amphisbaenidae) from juvenile to adult, which was discussed to reflect dietary conservation in burrowing worm lizards. Hipsley and Müller (2017) showed that the cranial shape of lacertid lizards varied significantly among biomes and showed substantial convergence among the ontogenetic trajectories of arid-dwelling lineages. Conversely, Piras et al. (2011) discussed that despite differences in diet among *Podarcis* lizards, the conserved ontogenetic trajectories are linked to phylogenetic signal.

Among snakes, Murta-Fonseca and Fernandes (2016) discussed the

importance of studies of ontogenetic allometry to diet in snakes by inspecting the skull shape of *Hydrodynastes gigas*. Among snakes, Silva et al. (2018) showed that the skull shape changes in the snake *Bothrops atro*x from neonate to adult in correspondence with shifts in their diet (from lizards to large mammals). Esquerré et al. (2017) showed that allometric coefficients are highly evolvable and that there is an association between ontogenetic allometry and habitat ecology among species of pythons and boas for similar ecology.

Ontogenetic allometry also allows for the investigation of heterochrony (Bhullar et al., 2012) and with large relevance for the understanding of macroevolution (McNamara & McKinney, 2005). Heterochrony can be defined as changes in the timing of onset and offset but also the rate of development relative to multivariate parameters (multivariate shape and centroid size) in ancestors (Klingenberg, 1998). Indeed, heterochrony is considered one of the most important biological phenomena that can generate evolutionary novelty (McKinney & McNamara, 1991; Hall, 1999). However, heterochrony does not provide the molecular mechanisms that explain time-regulated patterns, heterotopy, and other non-time regulated developmental processes (Zelditch, 2001). In this regard, knowledge of the molecular mechanisms and developmental processes of skull bone and shape development is limited for squamates, so one must first detangle the overall ontogenetic patterns.

Heterochrony was renewed as a research topic after an extensive and eloquent treatment of it by Stephen Jay Gould (1977) in his seminal book “Ontogeny and Phylogeny”. Gould reviewed in depth the parallel between ontogeny and phylogeny in the history of science, making it evident that it had long been acknowledged by scientists. Historically, the term heterochrony was introduced by Ernst Haeckel (1866) to recognize and explain a class of exception to his recapitulation theory. Heterochrony explained only phenotypic reversals (or, for him, return to primitive stages). Haeckel (1866)'s recapitulation theory, also known as the "biogenetic law", posited that the development of "derived" groups unfolded by added steps to "primitive" ones. The infamous sentence "ontogeny recapitulates phylogeny" is derived from this misleading understanding of the relationship between development and evolution. That view implied that every "advanced" organism would have gradually passed through its "primitive" phases throughout its lifetime (ontogeny). In other words, in their embryonic development, humans would have fish, amphibian, reptilian, and mammalian phases. The presence of pharyngeal slits would characterize the fish stage (gills) in our early embryos.

On the other hand, Von Baer's law of development (1828) opposed the notion of terminal addition and proposed that embryos within lineages diverged from a general morphological configuration to specialized outcomes towards their late development. The putative conserved stage became known as the phylotypic stage (Duboule, 1994; Richardson et al.,

1995; Galis & Metz, 2001), which is an oversimplification of the actual observed morphological variation (Hall, 1997; Richardson, 1997; Bininda-Emonds et al., 2003), and so should be seen as the phylotypic period instead (Richardson, 1997). The presence of a phylotypic period for amniotes has been shown based on the analyses of the shape of facial prominences, which also suggested that, instead of constrained, this phase is under the regime of negative selection that opposes the rise of facial clefts (Young et al., 2014). The conservation of gene expression at this developmental interval also supports the "developmental hourglass" model of phenotypic change throughout development (Kalinka et al., 2010; Irie & Kuratani, 2014). This model states that intermediate developmental periods (stages) are more conserved (phenotypic and molecular) than early or late developmental periods (stages) (Salvador-Martínez, 2016). Gould (1977) connected Von Baer's law of development with Darwin's theory of evolution by natural selection. The relevance of Von Baer's ideas has been recently re-stated to contemporary science (Abzhanov, 2013).

Gould (1977) rejected recapitulation in the Haeckelian sense but reiterated that there is a ubiquitous parallel between ontogeny and phylogeny, reframing heterochrony to a process-based approach linked with allometry and rates of development. A processual view of heterochrony had been, in fact, for example, anticipated by Gavin de Beer (1930, 1951) who considered both acceleration and retardation of development concerning sexual maturation as well as rates of growth. Gould's (1977) major innovation was the proposition of the "Clock Model", which could objectively describe and test different patterns of heterochrony through dissociated parameters: size, shape, and age. This model was later formalized to include the onset/offset of development, growth rates, and initial size (Alberch et al., 1979). This updated model provided clear graphical expectations of expected patterns of heterochrony in biological data when comparing descendent lineages to putative ancestral ones and clarified its terminology to dissociate it from Haeckelian definitions.

Heterochrony was classified into paedomorphosis (Figure 7: a, c, and e) and peramorphosis (Figure 7: b, d, and f) (Gould, 1977; Alberch et al., 1979). Paedomorphosis would occur due to truncation in the development time that could take place by a lower rate of development (neoteny), earlier maturation of adults (early offset) (progenesis), or late onset of development (post-displacement) in descendant lineages. Peramorphosis underlies the rise of new morphologies (novelty) due to an extended developmental period (delayed offset or hypermorphosis), an earlier onset of development (predisplacement), or an increased rate of development (acceleration) (Alberch et al., 1979; McNamara, 1986).

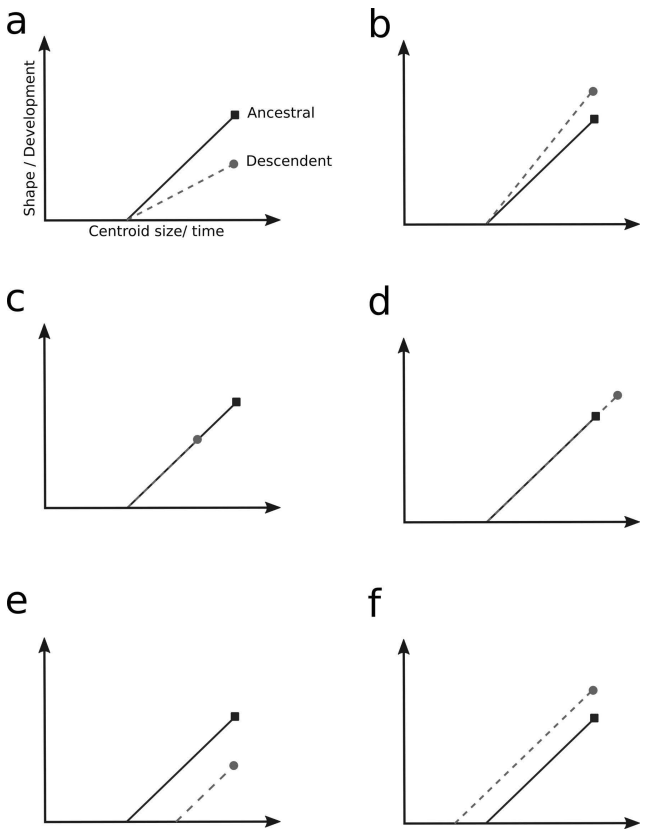

Figure 7 Heterochrony charts based on the regression of shape as a proxy for development onto centroid size as a proxy for developmental time. Solid line with a square tip depicts an ancestral adult and dashed line with a round tip the same stage but in the descendant lineage. Paedomorphosis: a (neoteny), c (progenesis), and e (postdisplacement). Peramorphosis: b (acceleration), d (hypermorphosis), and f (predisplacement).

These heterochony approaches were initially designed from a univariate notion of shape (ratios). Analyses and graphical outputs were expanded considering multivariate shape to distinguish patterns of undissociated-dissociated, trait-size relationships linked with heterochrony (Klingenberg & Spence, 1993). To evaluate heterochrony with multivariate data, the first stage is to inspect if ontogenetic trajectories are parallel in the ontogenetic morphospace (Mitteroecker et al., 2004). Mitteroecker et al. (2005) extrapolated that heterochrony would hardly be found for complex structures as parallel ontogenetic trajectories was a hard requirement.

If parallelism of trajectories was found, parameters of geometric shape (a proxy for a developmental change) could be regressed onto values

of centroid size (a proxy for developmental time), so generating regression plots with phenotypic trajectories (Mitteroecker et al., 2004; Mitteroecker et al., 2005). This is analogous to Alberch's approach (Figure 7), but conclusions of patterns of heterochrony should be cautious due to the multiple axes of variation that must be preferably integrated (Mitteroecker et al., 2004). Modern methods of comparisons of ontogenetic trajectories automatically quantify the shape of ontogenetic trajectories (direction, angle, and magnitude) and test their parallelism and heterochrony in a multivariate sense (Collyer & Adams, 2007; Collyer & Adams, 2013; Bhullar et al., 2012).

Considering the study of heterochrony in lizards, Raia et al. (2010) found parallel ontogenetic trajectories between populations of *Podarcis sicula* in insular and mainland environments, but young individuals in the inland population had a more advanced level of morphogenesis of the skull. Piras et al. (2011) verified that the developmental rates did not differ among four species of *Podarcis* but pointed out hypermorphosis. Hipsley and Müller (2017) found post-displacement in the development of desert lacertids, so they begin their development later than the time their ancestors would have done (the ancestral state was inferred based on biogeography and dispersion patterns). Gray et al. (2019b) did not find a clear pattern of heterochrony for most species of agamid lizards but divergence from juvenile skulls (similar shapes among species) to adult skulls (different shapes among species). The authors explained that divergent patterns of ontogenetic trajectories in agamid lizards are at least partially associated with habitat shifts.

Among snakes, peramorphosis and paedomorphosis were suggested based on the post-natal ontogenetic trajectories of only five species in comparison with two outgroup lizard species (Palci et al., 2016). This low number of samples would barely provide any resolution for such large claims and lacked a formal mathematical approach. Esquerré et al. (2017) identified hypermorphosis in the post-natal head development of a large dataset of pythons and boas in comparison to their estimated ancestral state (reconstructed from the slope values of allometric equations). Sherratt et al. (2019) found paedomorphosis (progenesis) in the postnatal development of the skull in burrowing sea snakes (Hydrophiinae) that prey on eels.

Previous to the first publication included in this dissertation (Da Silva et al., 2018), there was little knowledge of the quantitative development of the skull of lizards and snakes, particularly for prenatal development. A combined analysis of the ontogenetic trajectories had never been done, neither their connection to ecology on a large scale. Finally, the role of heterochrony was unclear.

2) AIMS

This doctoral research, detailed in this dissertation, aimed at producing the first large-scale macro-evolutionary study of the skull shape evolution of snakes and lizards (Squamata). This was achieved by using state-of-the-art technologies (High-Resolution Computed Tomography) and analytical approaches such as Geometric Morphometrics, cutting-edge methods of multivariate statistics and methods for ancestral state estimation. The analytical approach was interdisciplinary by integrating data from morphology, paleontology, ecology, phylogeny, and development.

2.1) MAIN RESEARCH GOALS

- Quantifying and comparing skull shape disparity to describe patterns of convergence and divergence potentially linked with natural selection, like expected convergences between fossorial lizards and snakes. Publications I and II.
- Analyzing the functional relationship between skull shapes and habitat ecologies in lizards and snakes in the hope to find a correlation that enables us to estimate the ancestral ecology of snakes from skull shape parameters. Publication I.
- Estimating the ancestral skull shape and size of snakes to shed light on the ongoing debate of the ecological origin of snakes. Publications I and II.
- Dissecting the relationship between natural selection and ontogeny in the rise and diversification of skull shapes of lizards and snakes, expanding our understanding of this relationship for amniote skull evolution. Publications I and II.

3) MATERIALS AND METHODS

3.1) MATERIAL: BIOLOGICAL SAMPLES

The number of skulls and species for two-dimensional (2D) and three-dimensional (3D) analyses are shown in Table 1 (Supplementary Tables 1 and 2 in Publication I, Additional file 2 in Publication II). *Highlighting, sampling covered all major extant and extinct lineages of lizards and snakes, a number never achieved before the study (I). The skull shape of fossils had never been analyzed with geometric morphometrics.*

Table 1 Information about samples.

Analyses	Samples	Lizards	Snakes	Outgroup	Total
2D (I)	Skulls (all samples)	233	171	4	408
	Species (adults)	173	131	1	304
	Fossils	19	7	1	27
	Fossil species	17	5	1	23
	Adult skulls	176	134	1	311
	Juvenile skulls	6	6	1	13
	Embryonic skulls	53	31	2	86
3D (I)	Skulls (all samples)	52	73	-	125
	Species (adults)	36	55	-	91
	Fossil skulls	-	-	-	-
	Fossil species	-	-	-	-
	Adult skulls	37	55	-	92
	Juvenile skulls	1	3	-	4
	Embryonic skulls	14	15	-	29
3D (II)	Skulls (all samples)	59	52	1	112
	Species (adults)	59	52	1	112
	Embryonic heads	27	-	-	8

Lateral view images of the skulls were mined from the literature and photographed at the host institute (Institute of Biotechnology) and the Museum für Naturkunde in Berlin. The types of 2D data included published accurate drawings of skulls as well as high-resolution photographs of dry, cleared-and-stained (alcian blue and alizarin red), and scanned samples by high-resolution computed tomography (CT). CT is a process that extracts anatomical information from bones by projecting a laser bin on a rotating sample inside of a CT-scan machine, so generating a stack of photos used for 3D reconstruction of the skull surfaces (Broeckhoven & du Plessis, 2018). This combination of sources of data had been successfully employed for geometric morphometrics (Bhullar et al., 2012). Sources of 3D skull data were obtained from the Digital Morphology Database (DigiMorph) and new CT scanned samples were generated at the University of Eastern Finland (Skyscan 1172 microCT), Museum für Naturkunde Berlin (Phoenix nanotom CT), and the University of Helsinki (Skyscan 1272 microCT and Phoenix Nanotom 180). The new samples were from the Tropicario Zoo in Helsinki, the Animal Facility of the Institute of Biotechnology in the University of Helsinki, Finnish Museum of Natural History (LUOMUS), Museum für Naturkunde Berlin (MZB), Museum of Comparative Zoology at Harvard (MCZ), and the American Museum of Natural History (AMNH).

A main composite phylogenetic topology was used for the comparative analyses by combining the most recent and inclusive molecular (Pyron, 2013) (I and II) and combined molecular and

morphological topologies (Reeder et al., 2015; Hsiang et al., 2015) (I). This approach had been previously employed for geometric morphometric studies (Bhullar et al., 2012).

3.2) METHODS

Table 2 Main references for theory and applications of employed methods.

Methods	References
2D and 3D Landmarking	Zelditch et al., 2004
Procrustes Superimposition	Zelditch et al., 2004
Principal Component Analysis	Zelditch et al., 2004
Thin-Plate Spline	Klingenberg, 2013a
Squared-Change Parsimony	Sidlauskas, 2008
Multivariate Regression Analysis	Klingenberg 2016
Multivariate Analysis of Variance (MANOVA)	Zelditch et al., 2004
Comparative methods	Monteiro, 2013
Phylogenetic signal	Adams, 2010
Estimation of Convergence	Stayton, 2015
Discriminant Function Analysis	Yi and Norell, 2015
Phenotypic Trajectory Analysis	Collyer and Adams, 2007
Analysis of Variance (ANOVA)	Zelditch et al., 2004

3.2.1) MORPHOLOGY: PROCRUSTES AND PCA (I/II)

In the 2D data, skull shape configurations were described by 20 landmarks and all skulls were scaled by pixel size based on a reference scale for each image in the software tpsDig v2.17 (Rohlf, 2013) (Publication I). Three-dimensional skull shape was described by the digitization of 61 and 65 landmarks (Publication I and II, respectively) on the rendered and segmented skull surfaces that were scaled by voxel size in the software Amira 5.5.0 (Stalling et al., 2005). The anatomical description of landmarks use terminologies described in Evans (2008), Cundall and Irish (2008), Olori and Bell (2012), Maisano and Rieppel (2007), and Gauthier et al. (2012). For more details, see Supplementary Tables 3 and 4 (Publication I) as well as Additional file 6 (Publication II). *Highlighting, study (I) was the first to apply this formal mathematical approach to quantify skull shape differences and similarities between snakes and lizards.*

The following geometric morphometric analyses were done in the package MorphoJ v1.06 (Klingenberg, 2010) unless otherwise stated. The Procrustes Generalized Least-Square method, here employed, became the norm for superimposition of landmark configurations (Rohlf & Slice, 1990).

It eliminates non-shape variation: scaling, translation, and rotation.

The procedure follows three steps (Zelditch et al., 2004). A) Centers each configuration of landmarks at the origin (subtracting coordinates of the centroid from the corresponding coordinates of each landmark). This translates the centroid of each configuration to the origin. The coordinates of the landmarks now reflect their deviation from the centroid. The centroid point is calculated as the mean of coordinates of all landmarks. B) Scale the landmarked configurations to unit centroid size by dividing each coordinate of each landmark by the centroid size. The centroid size is calculated as the square root of the sum of squared distances among all the landmarks to the centroid. C) Select one configuration to be the reference, then rotate subsequent configurations to minimize the sum of square distances between configurations of landmarks. It minimizes the partial Procrustes Distance, calculated by the square root of the sum of the squared differences between the coordinates of landmarks. This is done iteratively until full superimposition.

The interpretation of the shape space remains mathematically and visually complex after the superimposition procedure (Kendall et al., 1999), offering downstream analytical and interpretation challenges (Zelditch et al., 2004). Circumventing it, a projection of the original Kendall's shape space onto a Euclidean tangent space has become the norm (Rohlf, 1999). This approximation is a reasonable choice for biological variations, unlikely to result in significant distortions (Zelditch et al., 2004). To confirm, the deformation was checked by performing a regression through the origin for distance in tangent space onto Procrustes distance (in radians) in the software tpsSmall ver.1.29 (Rohlf, 2014). Finally, shape variables can be interpreted as axes for the linear tangent space and their scores treated in multivariate analyses (Adams et al., 2004).

The skull shape distribution was visualized using Principal Component Analysis of shape variation (PCA), an ordination method that depicts a low-dimensional representation of high-dimensional data such as landmark-based shape (Zelditch et al., 2004). Principal Components (PCs) are orthogonal and independent from each other. The first principal component (PC1) describes most of the skull shape variation in the data and other axes in progressively lesser proportions (Zelditch et al., 2004). Quantified samples are distributed in comparison to the mean shape in a PC1-PC2 plot and other combinations (Klingenberg, 2013a). This approach to visualize the distribution of shapes generates one type of morphospace (Mitteroecker & Huttegger, 2009).

PCA shows the distribution of shapes but does not describe them visually. Direct visualization of shape variation is considered a core advantage of geometric morphometrics (Klingenberg, 2013a). Indeed, the Thin-Plate Spline (TPS) Interpolation method generates partial warps that can be used as shape variables that are especially useful for graphical visualization of shape deformation by bending energy (Bookstein, 1989).

They formalized Thompson (1917)'s "Theory of Deformation". Wireframes show vectors departing from each landmark, depicting the direction (arrowhead) and magnitude (vector length) of shape changes from the mean shape. Landmarks can be connected by straight lines, curved outlines, or warped three-dimensional surfaces for better interpretation of biological shapes (Klingenberg, 2013a).

Evolutionary Allometry was quantified by the multivariate regression analysis of shape (Procrustes Coordinates) onto size (Log-centroid size) under 10.000 permutations for evaluation of statistical significance against the null hypothesis of total independence between the two variables (Monteiro, 1999). The residual score was used to calculate a new morphospace that was adjusted by size-correlated shape (Drake & Klingenberg, 2008). Large differences between the two morphospaces would indicate that evolutionary allometry is an important factor in skull evolution (Klingenberg et al., 2016).

3.2.2) ECOLOGY: COMPARATIVE METHODS AND CONVERGENCE (I)

The simplified habitat where each species locomotes and forages most frequently was mined from the literature: aquatic (includes freshwater, marine, and semi-aquatic species), terrestrial (surface locomotion and foraging, including saxicolous species), leaf-litter (terrestrial but living under vegetation layers or surface debris), fossorial (living and foraging mostly underground); and arboreal (adapted for locomotion between tree branches or bushes, including semi-arboreal species). References are available in Supplementary Tables 1 and 2 (Publication I).

A potential association between habitat and skull shapes (11 PCs) was tested using Phylogenetic Multivariate Analysis of Variance (MANOVA) (Garland et al., 1993) under a Brownian-motion model of evolution in the R-package geiger v2.0.667 (Harmon et al., 2008). The test shows if skull shapes are grouped by ecology, for instance, see also a previous application of the method that is relevant for our studies: Fabre et al. (2016). A significant association between shape and ecology allows ecologies to be predicted by shape parameters, including fossils, for instance, see also a previous application of the method that is relevant for our studies: Yi and Norell (2015).

Quantification of skull shape convergence was performed with a distance-based method known as C1–C4 and its statistical significance was assessed by 1000 evolutionary simulations according to a Brownian-motion model in the R-package convevol v1.1 (Stayton, 2015). C1 value is the most relevant proportion and is calculated from Equation (1), where *Dtip* is the distance (Euclidean or Procrustes) between the convergent species in phenotypic space and D_{max} is the maximum distance between members (extant, estimated ancestor) of each lineage that led to the tip specimens

that are being compared. C1 can have values of 0 to 1, where 0 indicates no convergence and 1 that the lineages became indistinguishable. *Highlighting, study (I) was the first to apply this formal approach to evaluate skull shape convergence between snakes and lizards.*

Equation 1 $C1 = 1 - D_{tip}/D_{max}$

3.2.3) PHYLOGENY: SIGNAL AND ANCESTRAL ESTIMATIONS (I /II)

The multivariate scores of the Most Recent Common Ancestors (MRCAs) were estimated using the unweighted algorithm of Squared-Change Parsimony (SCP) (Maddison, 1991). SCP minimizes the sum of squared changes along each branch of the phylogenetic tree. The ancestral value at each node is the mean of the three values around it (two child nodes + one parent node). Associated with PCA, the graphical outcome is known as a phylomorphospace, where Operational Taxonomic Units (OTU - tip specimens) are connected by lines to their estimated ancestral nodes (Sidlauskas, 2008). Lines depict evolutionary branches and so evolutionary trajectories as described by PCs (Sidlauskas, 2008). Ancestral skull sizes were reconstructed in the same way and visualized by mapping the centroid sizes onto phylogeny. (Publications I and II).

The phylogenetic signal was calculated using a multivariate generalized *K*-statistics in the R-package geomorph v3.0.565 (Publications I and II) (Adams & Otárola-Castillo, 2013). Adams (2010) developed a multivariate generalization of the *K* statistic of Blomberg et al. (2003) that is useful for quantifying and evaluating phylogenetic signals without ancestral estimation. Values of *K* range from $0 \rightarrow \infty$, with an expected value of 1.0 under Brownian motion or neutral evolution. Values of $K<1.0$ describe data with less phylogenetic signal than expected, meaning that grouping of species does not follow their taxonomic relationship - an initial indication of convergent trends; and values of $K>1.0$ describe data with greater phylogenetic signal than expected under the Brownian model of evolution and speciation.

Finally, Discriminant Function Analysis (DFA) is an ordination method used to find the shape that separates two groups and to classify unknown samples into an ecological group based on a training set of shape parameters where the group membership is known a priori (Fisher, 1936). For instance, see previous application of the method that is relevant for our studies in Yi and Norell (2015). Discriminant Function Analysis for the prediction of the ancestors was done in the R-package MASS v7.3-47 (Venables & Ripley, 2002). *Highlighting, study (I) was the first to apply this formal approach to estimate the ancestral ecology of snakes from their skull shape parameters.*

3.2.4) DEVELOPMENT: TRAJECTORIES AND RATES (I/II)

An ontogenetic morphospaces was plotted to inspect similarities and differences among the ontogenetic trajectories between lizards and snakes (I). Each ontogenetic sample was connected by lines in a chronological time (or developmental stage) so they would form a series of ontogenetic "stages". Phenotypic Trajectory Analysis (PTA) was performed to quantify the geometric properties of the ontogenetic trajectories (path length, direction, and angles) between lizards and snakes in the morphospace (Collyer & Adams, 2013; Adams & Collyer, 2009). Intermediate stages are not easily comparable, so the initial and final developmental samples of each trajectory were embryos at stage 10 (the last one before hatching) and mature adults, respectively. The *trajectory.analysis* function available in geomorph v3.0.5 was used to compare the ontogenetic trajectories (Adams et al., 2013). Statistical significance was accessed by a random permutation procedure of 1000 iterations.

PTA tests vectors of phenotypic change in terms of two important attributes: the magnitude of change (vector length) and the direction of change described by trait covariation (the angular difference between vectors) (Collyer & Adams, 2007). It is appropriate for dynamic data that has at least two states such as ontogeny. PTA calculates the difference in mean values of shape vectors, where the mean vector shape (from PC scores) at embryonic stage 10 (j) is subtracted by the mean vector shape of adults (k) as shown in Equation 2. This was calculated for the two groups: snakes (i = 1) and lizards (i = 2).

Equation 2 $$\Delta\bar{\mathbf{Y}}_i = \bar{\mathbf{Y}}_{ij} - \bar{\mathbf{Y}}_{ik}$$

The magnitude of differences between the two means is calculated by the Euclidean Distance (D_{ei}), returning the length of trajectories. To discern if the level of phenotypic change of one group is greater than another, the test statistic D_{e1} - D_{e2} with permutations is performed.

The difference in direction between ontogenetic trajectories is calculated by their vector correlation or the inner product of the two vectors scaled to unit length as shown in Equation 3. The *arccosine* of VC yields an angle Theta (θ), with a minimum value of 0 if the vectors are parallel and a maximum value of 90 if they are orthogonal.

$$\mathrm{VC} = \left(\frac{\Delta\bar{\mathbf{Y}}_1}{D_{\mathrm{E}_1}} \cdot \frac{\Delta\bar{\mathbf{Y}}_2^{\mathsf{T}}}{D_{\mathrm{E}_2}}\right)$$

Equation 3

Analysis of geometric attributes of ontogenetic trajectories can be used to test for heterochrony (Mitteroecker et al., 2005). Multivariate regression of Procrustes shape (a proxy of development) onto Log-centroid size (a proxy of time) was performed (Klingenberg, 2016; Bhullar et al.,

2012). PTA was used again to quantify the slope, length, and angle of vectors of ontogenetic skull shape change between a pool of evolutionary descendant trajectories (snakes) against a pool of evolutionary ancestral trajectories (lizards) (Collyer & Adams, 2013). This time, the Regression Scores were used as shape parameters (Bhullar et al., 2012). *Highlighting, study (I) was the first to apply this formal approach to evaluate ontogenetic skull shape changes and heterochrony between snakes and lizards.*

The total duration of embryonic development was compared among 126 squamate species through Analysis of Variance (ANOVA) (Table 3). The incubation temperature and gestation periods had been previously compiled by Köhler and Haecky (2005). ANOVA tested if the developmental time of lizards and snakes was equivalent or significantly different.

In complement, the degree of ossification of both parietal and frontal bones was coded and compared among 35 different snake and lizard species at a pre-hatchling embryonic stage (stage 10 of *Boaedon fuliginosus*), serving as a marker of the offset of skull development. The parietal and the frontal bones are the last ones to complete ossification (Maisano, 2001), so are excellent markers of the completion of skull bone development. (See also Supplementary Table 14 in Publication I). Finally, I coded head traits for early embryos at 0-1 dpo (days post-oviposition). More information on samples, comparisons, and coding systems in Figure 2C/Datasheet 9 in Publication II.

Table 3: List of species and their respective length of embryonic development measured in a controlled temperature of incubation (30+/-1 Celsius). This table was not added to Publication I. All the other tables are shown in the original publications.

Group	Species	Family	Temp. (0C)	Length (days)
Lizard	*Acanthocercus atricollis*	Agamidae	30	75
Lizard	*Agama impalearis*	Agamidae	30	54
Lizard	*Calotes versicolor*	Agamidae	30	37
Lizard	*Chlamydosaurus kingii*	Agamidae	29.5	62.5
Lizard	*Ctenophorus decresii*	Agamidae	30.5	59
Lizard	*Draco spilopterus*	Agamidae	29	36
Lizard	*Hydrosaurus amboinensis*	Agamidae	30	78.5
Lizard	*Paralaudakia caucasia*	Agamidae	30	72
Lizard	*Leiolepis guttata*	Agamidae	30	64
Lizard	*Phrynocephalus mystaceus*	Agamidae	30	61
Lizard	*Physignathus cocincinus*	Agamidae	30	101
Lizard	*Pogona minor*	Agamidae	30	54

Lizard	*Trapelus mutabilis*	Agamidae	30	61
Lizard	*Tympanocryptis tetraporophora*	Agamidae	30	48
Lizard	*Uromastyx acanthinura*	Agamidae	30	99.5
Lizard	*Pseudopus apodus*	Anguidae	30	52
Lizard	*Chamaeleo africanus*	Chamaeleonidae	30	183
Lizard	*Furcifer antimena*	Chamaeleonidae	30	360
Lizard	*Basiliscus basiliscus*	Corytophanidae	30	98
Lizard	*Laemanctus longipes*	Corytophanidae	30	64
Lizard	*Crotaphytus collaris*	Crotaphytidae	30	86
Lizard	*Gambelia wislizenii*	Crotaphytidae	30	60
Lizard	*Anolis bimaculatus*	Dactyloidae	30	43
Lizard	*Coleonyx brevis*	Eublepharidae	30	70
Lizard	*Hemidactylus mabouia*	Eublepharidae	30	60
Lizard	*Chondrodactylus angulifer*	Gekkonidae	30	85
Lizard	*Geckolepis typica*	Gekkonidae	30	45
Lizard	*Hemidactylus brookii*	Gekkonidae	30	55
Lizard	*Homopholis mulleri*	Gekkonidae	30	80
Lizard	*Lepidodactylus lugubris*	Gekkonidae	30	100
Lizard	*Lygodactylus pictus*	Gekkonidae	30	78
Lizard	*Pachydactylus tsodiloensis*	Gekkonidae	30	49
Lizard	*Phelsuma borbonica*	Gekkonidae	30	64
Lizard	*Stenodactylus sthenodactylus*	Gekkonidae	30	80
Lizard	*Uroplatus phantasticus*	Gekkonidae	30	61
Lizard	*Broadleysaurus major*	Gerrhosauridae	30	77
Lizard	*Neusticurus bicarinatus*	Gymnophthalmidae	30	86
Lizard	*Heloderma horridum*	Helodermatidae	30	170
Lizard	*Conolophus subcristatus*	Iguanidae	30	105
Lizard	*Ctenosaura bakeri*	Iguanidae	29.5	90.5
Lizard	*Cyclura collei*	Iguanidae	29.5	85
Lizard	*Iguana delicatissima*	Iguanidae	30	119.5
Lizard	*Sauromalus ater*	Iguanidae	29.5	83.5
Lizard	*Gallotia galloti*	Lacertidae	30	90
Lizard	*Lacerta agilis*	Lacertidae	29.5	34
Lizard	*Darevskia armeniaca*	Lacertidae	30	55
Lizard	*Archaeolacerta bedriagae*	Lacertidae	30	40
Lizard	*Dinarolacerta mosorensis*	Lacertidae	30	19
Lizard	*Dalmatolacerta oxycephala*	Lacertidae	30	49
Lizard	*Parvilacerta parva*	Lacertidae	30	33
Lizard	*Omanosaura jayakari*	Lacertidae	30	94
Lizard	*Podarcis muralis*	Lacertidae	30	27
Lizard	*Teira dugesii*	Lacertidae	30.5	51
Lizard	*Timon lepidus*	Lacertidae	30	88
Lizard	*Petrosaurus thalassinus*	Phrynosomatidae	29.5	56
Lizard	*Phrynosoma asio*	Phrynosomatidae	30	80.5
Lizard	*Sceloporus scalaris*	Phrynosomatidae	30	44

Lizard	*Uta stansburiana*	Phrynosomatidae	30	47
Lizard	*Asaccus platyrhynchus*	Phyllodactylidae	30	48
Lizard	*Ptyodactylus hasselquistii*	Phyllodactylidae	30	85.5
Lizard	*Tarentola angustimentalis*	Phyllodactylidae	30	87
Lizard	*Polychrus marmoratus*	Polychrotidae	30	133
Lizard	*Ctenotus taeniolatus*	Scincidae	30	40
Lizard	*Bassiana duperreyi*	Scincidae	30	29
Lizard	*Eumeces algeriensis*	Scincidae	30	47
Lizard	*Lampropholis guichenoti*	Scincidae	30	27.5
Lizard	*Morethia adelaidensis*	Scincidae	30	29
Lizard	*Scincus scincus*	Scincidae	29.4	64
Lizard	*Gonatodes albogularis*	Sphaerodactylidae	30	72
Lizard	*Saurodactylus mauritanicus*	Sphaerodactylidae	30	60
Lizard	*Teratoscincus microlepis*	Sphaerodactylidae	30	75
Lizard	*Tupinambis teguixin*	Teiidae	30	171
Lizard	*Varanus bengalensis*	Varanidae	30	170
Snake	*Calabaria reinhardtii*	Calabariidae	30.5	37.5
Snake	*Boiga dendrophila*	Colubridae	30	92
Snake	*Hemorrhois hippocrepis*	Colubridae	30	66
Snake	*Coniophanes fissidens*	Colubridae	30	53
Snake	*Coronella girondica*	Colubridae	30	51
Snake	*Dasypeltis scabra*	Colubridae	30	90
Snake	*Dipsas articulata*	Colubridae	30	85
Snake	*Orthriophis cantoris*	Colubridae	30	102
Snake	*Elaphe quatuorlineata*	Colubridae	30	58
Snake	*Farancia abacura*	Colubridae	30	57
Snake	*Gonyosoma oxycephalum*	Colubridae	30	115
Snake	*Heterodon nasicus*	Colubridae	30	59
Snake	*Lampropeltis getula*	Colubridae	30	75
Snake	*Erythrolamprus poecilogyrus*	Colubridae	30	93
Snake	*Liopholidophis dolicocercus*	Colubridae	30	59
Snake	*Coluber flagellum*	Colubridae	30	79
Snake	*Natrix matrix*	Colubridae	30	63
Snake	*Philodryas patagoniensis*	Colubridae	30	59
Snake	*Pituophis lineaticollis*	Colubridae	30	68
Snake	*Ptyas mucosa*	Colubridae	30	60
Snake	*Rhabdophis tigrinus*	Colubridae	30	47
Snake	*Rhinocheilus lecontei*	Colubridae	30	68
Snake	*Sonora semiannulata*	Colubridae	30	56
Snake	*Spalerosophis diadema*	Colubridae	30	84
Snake	*Spilotes pullatus*	Colubridae	30	56
Snake	*Telescopus fallax*	Colubridae	30	71
Snake	*Thrasops jacksonii*	Colubridae	30	84.5
Snake	*Aspidelaps scutatus*	Elapidae	30	67
Snake	*Bungarus caeruleus*	Elapidae	29.5	60

Snake	*Cacophis squamulosus*	Elapidae	30	74
Snake	*Demansia vestigiata*	Elapidae	30	67
Snake	*Dendroaspis angusticeps*	Elapidae	30	107
Snake	*Naja haje*	Elapidae	30	77
Snake	*Oxyuranus scutellatus*	Elapidae	30	71.5
Snake	*Pseudechis australis*	Elapidae	30	68
Snake	*Pseudonaja modesta*	Elapidae	30	61
Snake	*Vermicella intermedia*	Elapidae	30	59
Snake	*Boaedon fuliginosus*	Lamprophiidae	30	60
Snake	*Malpolon monspessulanus*	Lamprophiidae	30	60
Snake	*Xenocalamus transvaalensis*	Lamprophiidae	30	55
Snake	*Pareas carinatus*	Pareatidae	30	62
Snake	*Aspidites melanocephalus*	Pythonidae	30	58
Snake	*Bothrochilus albertisii*	Pythonidae	30	68
Snake	*Leiopython albertisii*	Pythonidae	30	60
Snake	*Simalia boeleni*	Pythonidae	30	71
Snake	*Liasis fuscus*	Pythonidae	30	52
Snake	*Morelia amethistina*	Pythonidae	30.5	85
Snake	*Python curtus*	Pythonidae	30.5	65.5
Snake	*Malayopython reticulatus*	Pythonidae	30	87.5
Snake	*Anilios australis*	Typhlopidae	29.5	58
Snake	*Cerastes cerastes*	Viperidae	30	48
Snake	*Lachesis muta*	Viperidae	30	61
Snake	*Macrovipera lebetina*	Viperidae	30	42
Snake	*Pseudocerastes fieldi*	Viperidae	30	17
Snake	*Xenopeltis unicolor*	Xenopeltidae	30	76

4) RESULTS

Figure 8 summarizes the results obtained in this dissertation.

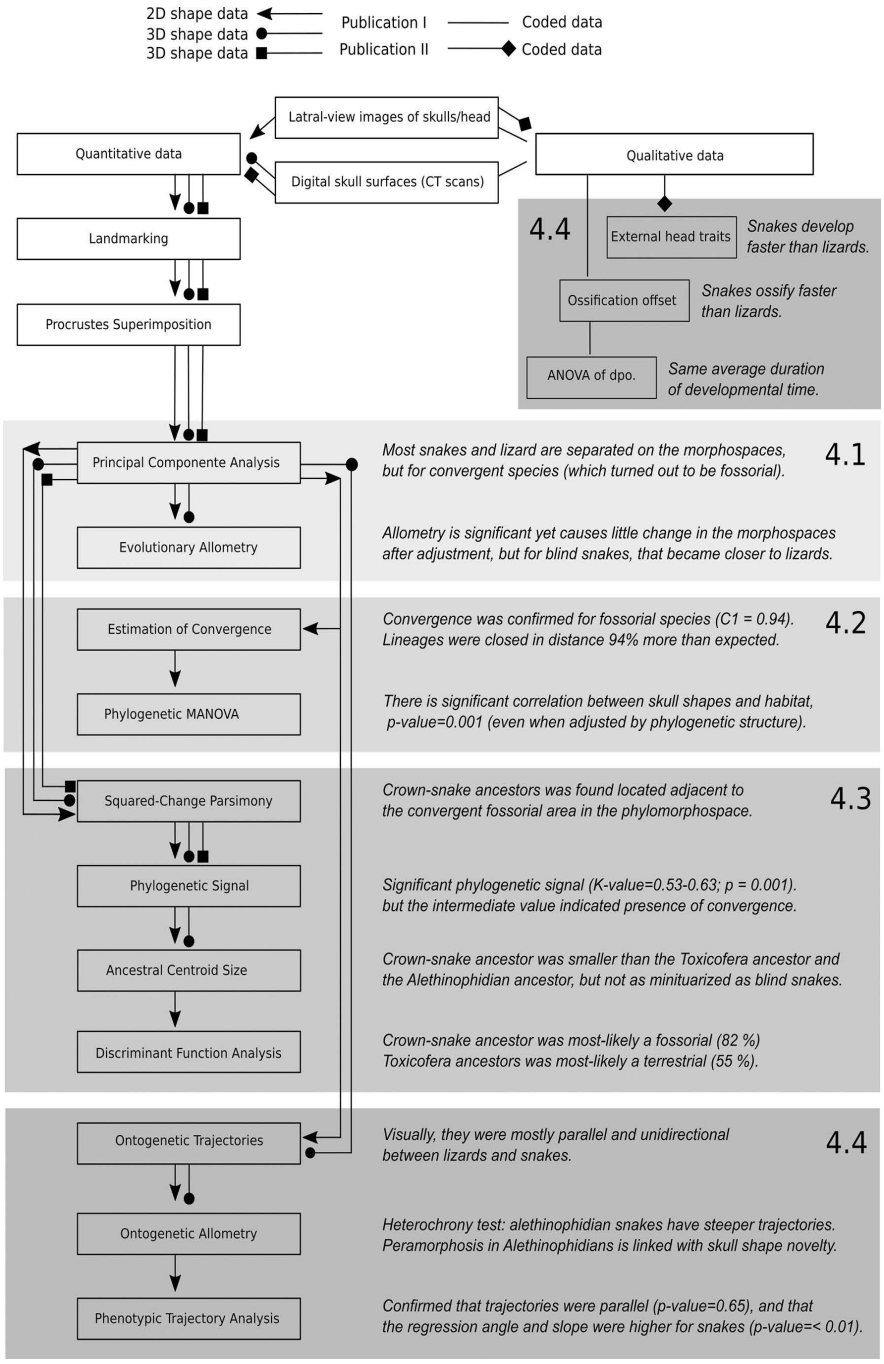

Figure 8 Summary of analyses and results by sections 4.1-4.4 divided by boxes colored in shades of gray. Lines with an arrowhead and circular tip show the workflow of the 2D and 3D data, respectively, in the publication I. Lines with a square tip show the workflow for 3D data in the publication II. Lines without a shaped tip represent the workflow for qualitative (coded) data in the publication I whereas lines with a diamond tip indicate coded data for publication II. The type of data (quantitative and qualitative) and their respective analyses (e.g., PCA) are delimited by rectangles with dark borders. Section 4.4 was divided into two: quantitative and qualitative.

4.1) MORPHOLOGY: DISPARITY IN THE MORPHOSPACE. (I/II)

This doctoral research departs from this first question: "Can skull shape converge between lizards and snakes?", and the first hypothesis: "Skull shape convergence evolved between fossorial lizards and snakes." Previous qualitative studies of the snake and lizard skulls had indicated morphological similarities between the skull and body of fossorial lizards (e.g., amphisbaenians and *Dibamus)* with fossorial snakes (e.g., scolecophidians and *Cylindrophis*) (Rieppel, 1993; Evans, 2008; McDowell, 2008; Cundall & Irish, 2008; Gans & Montero, 2008). Examples of skull shape convergence within snakes and lizards, including the underlying theory, are available in the sections 1.2.2 (general) as well as 1.4.2.1 and 1.4.2.2 (detailed). Moreover, convergence of skull traits had been already suggested to be a major source of phylogenetic uncertainty between lizards and snakes, affecting the interpretation of the ecological and phylogenetic origin of snakes (see section 1.3.3). Taking all those information into account, I analyzed the skull shape disparity of snakes and lizards by using two-dimensional geometric morphometrics (I).

The deformation of the skull shape space was not significant ($P<0.0001$) after projection onto the Euclidean tangent space, granting subsequent macroevolutionary interpretation and analyses.

PC1 and PC2 represents for more than 60% of the total skull shape variation in both 2D and 3D skull shape analyses. The morphospace occupation differed between lizards and snakes along with values of PC1 but for one region between (+) PC1 and (-) PC2, where species from distant lineages of both groups were found near each other (Figure 9). This is not expected under the assumption of neutral evolution. I implied that grouped skull shapes were likely convergent. Natural selection would have likely directed their similarities due to common ecological requirements. Most lizards were distributed along with values of PC2 and (+) PC1. Scolecophidian snakes were restricted to the putative convergent area. Alethinophidian snakes occupy a new area of the morphospace towards (-) PC1. Noticed that all snake fossils are near the other alethinophidian snakes. All fossil lizards were limited to the lizard area of the morphospace.

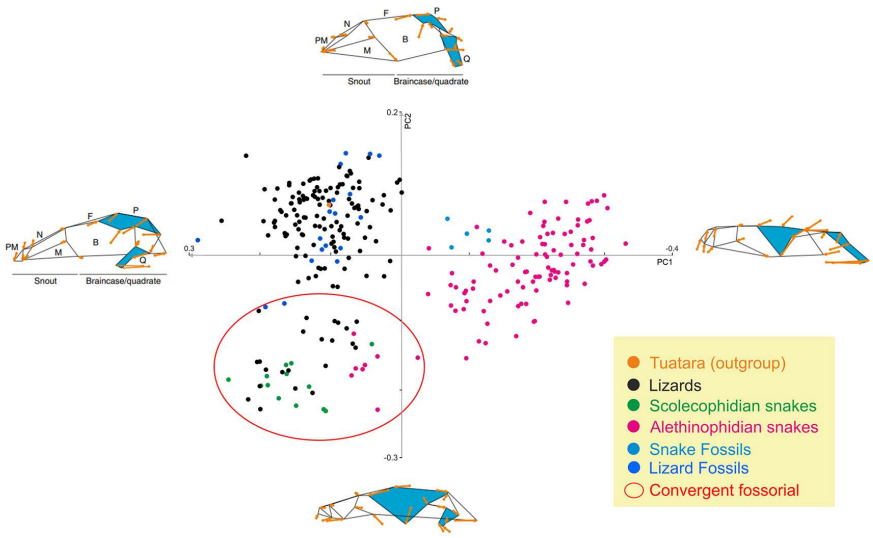

Figure 9 Morphospace shows the skull shape distribution of lizards (black dots), scolecophidians (green dots), alethinophidians (pink dots), snake fossils (light blue dots), lizard fossils (dark blue dots), and tuatara (outgroup, orange dot) from 2D analysis (Publication I). The shape wireframes shown at each extreme were estimated from extant adults. The orange arrows are the vectors of shape change (the arrowhead indicates its direction and the vector length its intensity from the mean shape). The large red circle shows the convergent area occupied by fossorial species.

(+) PC1 values represent skulls with variable shapes, but at the extreme values are located skulls with a tall skull rooftop due to an elongated parietal crest (e.g., Chamaeleonidae and Phrynosomatidae), triangular snout, prominent ocular region, and the ventral articular surface of the quadrate bone is projected towards the snout. This area is occupied mostly by lizards, but also some of the fossorial snakes towards lower values.

(-) PC1 values represent skulls with an elongated profile, and at its extreme lower values, the parietal recovers the lateral side of the braincase, the snout is more rounded, the ocular region is not as prominent, and the quadrate has its ventral articular surface projected towards the body and has an extremely elongated shaft. This area is exclusively occupied by alethinophidian snakes and snake fossils (*Dinilysia*, *Wonambi*, *Yurlunggur*, *Pachyrhachis*, and *Haasiophis*) towards "lower" values. *Highlighting, the study (I) was the first to employ geometric morphometrics to study squamate skull fossils.*

(+) PC2 values represent skulls of lizards and snakes, but at the

extreme values where only lizards are present, the skull has a tall skull rooftop with reduced parietal, the snout has a triangular profile but with an expanded maxillary bone, the ocular region is extremely prominent, and the quadrate bone is slightly anteroposteriorly projected and has a long shaft. This area is occupied by mosasauroids and Iguanidae. Other lizards occupy lower values.

(-) PC2 values represent skull of lizards and snakes, that extreme levels show a dorso-ventrally compressed skull (giving them a cylindrical profile) with an expanded parietal bone, the snout is compressed concerning the rest of the skull, the ocular region is highly reduced, and the quadrate bone is slightly posteroanterior projected and has a short shaft. This area is occupied by lizards of the families Rhineuridae, Bipedidae, Trogonophiidae, Amphisbaenidae, Scincidae, Gymnopthalmidae, Dibamidae, Pygopodidae, Anguidae, two rhineurid fossils - *Spathorhynchus natronicus* and *Plesiorhineura hatcherii,* the paraphyletic group "Scolecophidia", and some alethinophidian snakes of the families Anomochilidae and Uropeltidae.

Two-dimensional analysis of shape has limitations: landmarks do not cover the entire skull and gathering of images can introduce error. The 3D data largely circumvents them due to its digital nature. I then expanded analyses with three-dimensional configurations of landmarks (I and II). Patterns of skull shape disparity were found similar between data types (compare Figure 9 and Figure 10). *Highlighting, studies (I and II) pioneered 3D skull shape comparison between lizards and snakes.*

Finally, I found significant correlations between size and shape (p-values < 0.0001) (I), but evolutionary allometry had small effects on the total pattern (described 5%-15% of total shape variation) (I and II). The adjusted morphospace showed similar patterns, but for scolecophidians, now relocated near to non-fossorial lizards (or towards positive values of PC2).

Altogether, these findings indicated that similar skull shape solutions can be recurrently achieved, regardless of allometry. It is less likely that skulls of distantly related lineages would group so often by neutral evolution. Convergent evolution due to natural selection is more plausible, supporting the posed hypothesis, but subsequent formal analyses would be required.

4.2) ECOLOGY: SKULL FORM AND FUNCTION (I)

Next, I investigated the second question: "Is there a correlation between skull shape and habitat ecology?", and the second hypothesis: "Distinct skull shapes are associated with respective ecological functions so that ecologies could be estimated from skull shape parameters.", which partially derived from the above findings of putative convergence. Moreover, this hypothesis stems from the published literature, which

previously showed for limited groups, thus not squamate altogether, that ecology can influence and be influenced by skull shape in an association, or even, in some cases, in a determinist fashion (section 1.4.2.2). Thus, observed shape-ecology association at more inclusive evolutionary levels could be strong enough to have given risen to more comprehensive macroevolutionary patterns.

I plotted the ecologies of tip specimens onto the morphospace to further understand the relationship between skull shape and the habitat where each species most-frequently locomotes (categories and their definition are described in the material and methods) (I). I found that several fossorial lizards and snakes were grouped (Figure 10).

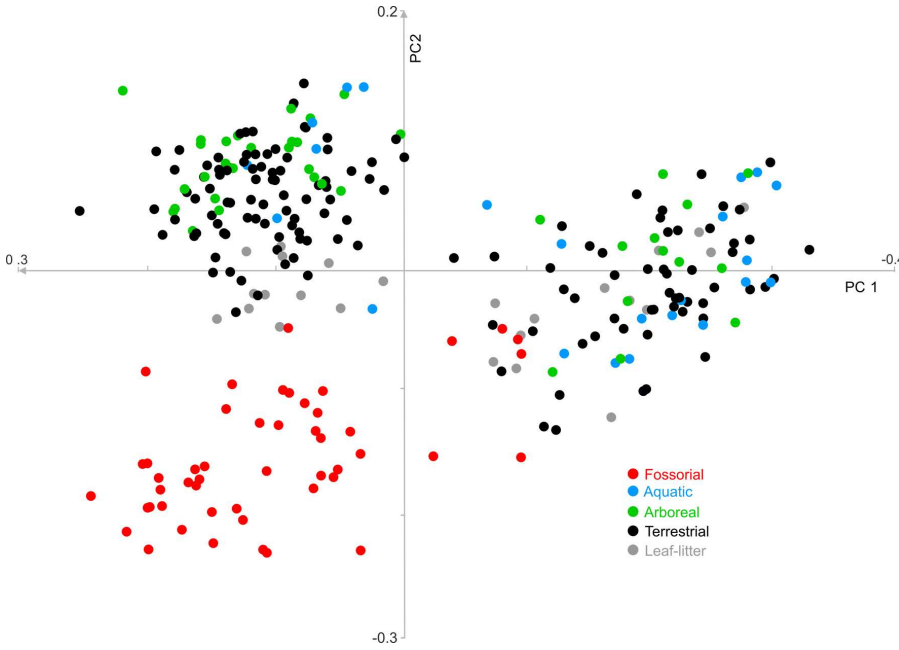

Figure 10 Morphospace shows the skull shape distribution and their respective habitat ecologies (Publication I). Ecologies are coded by different colors. Notice that several fossorial lizards and snakes are grouped between values of (+) PC1 and (-)PC2.

Among lizards, one can notice a partial gradient of ecologies fossorial - leaf litter - terrestrial - arboreal. Aquatic species are more scattered. For snakes, one can see clear differences between most fossorial species and the other ecologies. Multiple ecologies are represented among alethinophidians whereas scolecophidians are fossorial.

These patterns indicated that convergence was most likely presented between fossorial species of lizards and snakes and that ecology was

associated with differences in skull shapes. Indeed, the measure of convergence *C1* showed that macroevolution has closed (neared) 94% of the distance between fossorial species than would be expected by chance under a Brownian Motion model of evolution (I). Moreover, I observed a significant correlation between habitat on skull shapes (nsim=1000, F=7.5, p-value=0.001) by conducting a MANOVA test that was adjusted by the phylogenetic structure (I).

These findings demonstrated that shape parameters could be confidently used to estimate ancestral ecologies, supporting our hypothesis.

4.3) PHYLOGENY: ANCESTRAL ECOMORPHOLOGIES. (I /II)

The above ecological results provided the foundation to evaluate the third question: "What are the ancestral skull shapes and ecologies of snakes?" and current main ecological hypotheses: "snakes evolved from a fossorial ancestor", "snakes evolved from a marine ancestor", or "snakes evolved from a terrestrial ancestor". Those hypotheses were based on the vast literature, mostly focused on qualitative traits and phylogenetic inferences, although some had used geometric shapes of other structures, as presented in detail in the section 1.3 and underlying subsections.

I generated a phylomorphospace to estimate the shape of the MRCA of Toxicofera, the MRCA of crown snakes, and the MRCA of Alethinophidian snakes (I and II). This time, I describe the results for the 3D data, because it has fewer species and that facilitates the visualization of the evolutionary patterns. I found that the ancestor of crown snakes was recovered near the putative convergent region. The Toxicofera ancestor was found among other lizards (Figure 11). None of the snake and lizard fossils were positioned near the estimated ancestors of snakes. *Highlighting, the geometric skull shape of snake ancestors had never been estimated before the study (I).*

I found a significant phylogenetic signal (I: K-value = 0.53; p-value = 0.001 and II: *K*-value = 0.63; p-value = 0.001). Importantly, the intermediate values of *K* were indicative of large deviations of observed patterns from neutral evolution. This is compatible with confirmed convergent results in previous sections.

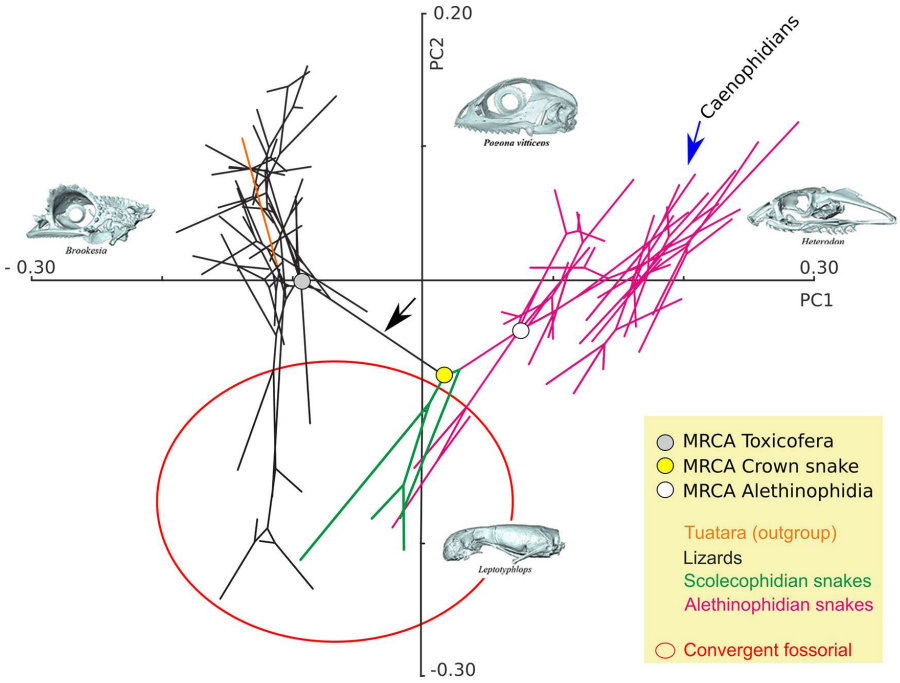

Figure 11 This Phylomorphospace was created by projecting the squamate phylogeny onto the morphospace delimited by the first two PC axes from the 3D analyses (Publication II). Major lineages and ancestral nodes are colored. The estimated lizard-to-snake transition took place between MRCA of Toxicofera (grey node) and MRCA of Crown Snakes (yellow node), as indicated by a black arrow. The red circle indicates the convergent shape space of fossorial species of lizards and snakes. 3D-rendered skulls show the extreme species for each axis in the phylomorphospace. The blue arrow shows the skull shape radiation among Caenophidian snakes that are most likely linked with increased macrostomy underlined by shape changes in the quadrate bone: elongation and anteroposterior projection of its ventral articular surface.

I employed a linear DFA with a cross-validation procedure to predict the ancestral ecologies of the estimated ancestral skull shapes, following previous results of a strong association between skull shape and habitat (I). I retrieved a fossorial ecology with 82% of likelihood for the MRCA of crown snakes and a terrestrial ecology for the MRCA of Toxicofera with 55% of correct assignment. In pairwise comparisons, fossorial forms were significantly different from all the other ecologies (p-value <0.001), also terrestrial from marine (p-value <0.001). Thereafter, a marine origin for snakes can be refuted, particularly for the crown snake ancestor, while a terrestrial-to-fossorial evolutionary change is favored. *Highlighting, this approach had never been employed for the skull regarding snake origin.*

There is a clear association between shape evolution and skull

innovation and radiation among snakes that occupy values of (-) PC1 and (+) PC2, particularly Caenophidian snakes, but multiple reversals of skull shape also did take place (e.g., *Cylindrophis*). Among other complex features of the skull, this trend is best represented by an increased elongation of the shaft of the quadrate bone as well as an anteroposterior projection of its ventral articular surface. This phenotype reached extreme shapes among caenophidian snakes, pythons, and boas (Figure 9 and 11).

Finally, I estimated the ancestral skull sizes (I). I found that the MRCA of crown snakes was smaller than the MRCA of Alethinophidian snakes (2D or 3D) and the Toxicofera (3D) (Figure 11). Moreover, Alethinophidian snakes diversified into a broad range of sizes - from miniaturized species such as *Rhinophis* (total skull size in mm) to large pythons (total skull size in cm) (Figure 12). This is best explained by reversals linked with fossoriality.

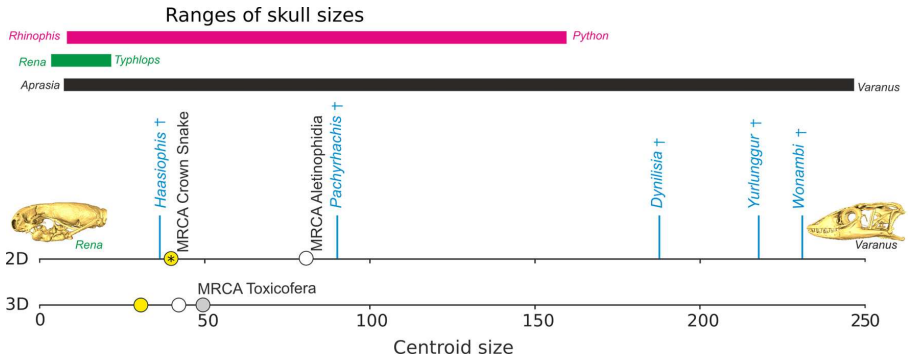

Figure 12 The centroid size variation (mm) of skulls for both extant and fossil species were traced onto the phylogeny, and ancestral estimation was done with squared-change parsimony (Publication I). The centroid size range of lizards, scolecophidians, and alethinophidians are shown as colored bars. The name of the smallest and largest species is shown for each group in the size distribution. The skull size of fossils and ancestors are indicated by lines with their names and colored circles, respectively. 3D-rendered skulls of *Rena* and *Varanus* show shapes for extreme sizes. * Indicates that for the 2D data the skull size of the MRCA of crown snakes and Toxicofera was only slightly distinct.

These findings demonstrated a new scenario for the early evolution of snakes from lizards: terrestrial to fossorial. This was a surprising finding, rejecting a marine-to-marine, fossorial-to-fossorial, or terrestrial-to-terrestrial scenario. They also indicated that the skulls of Alethinophidian snakes can be considered an evolutionary novelty.

4.4) DEVELOPMENT: HETEROCHRONY AND NOVELTY (I/II)

Alethinophidian skulls seem to represent a key innovation in terms of shape based on results shown above. I then sought potential underlying developmental processes and mechanisms. My fourth question was: "Is there a relationship between development and ecology in the evolution of snake skulls?", and the fourth hypothesis emerged: "Heterochrony underlies skull shape innovation". As shown in section 1.4.2.4, heterochrony is a common phenomenon involved in skull innovation and had been suggested for the snake skull evolution (e.g., Irish, 1989; Werneburg & Sánchez-Villagra, 2015) but never tested in comparison to lizards using skull shape and formal analyses.

I quantified and compared the geometric properties (length, direction, and angle) of phenotypic trajectories that were generated by vectors of shape changes between embryos and adults in the ontogenetic morphospace (I). I found that the phenotypic trajectories were largely conserved in length (Lizards: 0.109, Snakes: 0.11; p-value=0.94), angle (pairwise angle: 43.683; p-value=0.65), and direction between lizards and snakes. Thus, they are statistically parallel and unidirectional. These are prerequisites for subsequent heterochrony tests. *Highlighting, the study (I) provided the first comparison of ontogenetic trajectories between lizards and snakes.*

I quantified and compared the regression slopes as well as angles of the ontogenetic trajectories between lizards and snakes. This is a type of heterochrony test. I found that the ontogenetic trajectories of snakes had a significantly higher angle (Snakes: 10.58 +/- 3.65, Lizards: Mean=3.24 +/- 2.56; p-value < 0.01) and slope (Lizards: 0.06 +/- 0.05, Snakes: 0.19 +/- 0.07; p-value=< 0.01) (I) (Figure 13). This pattern fits the expectation of peramorphosis and the developmental mechanism of acceleration. This developmental process and mechanism would have allowed snakes to occupy novel skull shapes areas in the morphospace, which seemly conferred them new ecological functions. *Highlighting, peramorphosis due to acceleration had never been reported for snakes before the study (I).*

The shape transition from embryos to adults is complex, but a particularly striking modification is the elongation of the shaft of the quadrate bone and an anteroposterior projection of its ventral articular surface as the embryo ages. The same trends are seen in postnatal development. This ontogenetic transition is more pronounced among Alethinophidian snakes than in blind snakes and lizards. Those transformations reach novel shapes for Pythonidade, Boidae, Viperidae, Elapidae, Colubridae, and Lamprophiidae (Figure 11 – *Bitis*).

I compared the total length of the embryonic development of a large sample of oviparous squamates incubated in the same temperature range (~30 Celsius). ANOVA was not significant (p-value= 0.22), indicating that ontogenetic changes between lizards and snakes were due to rate instead.

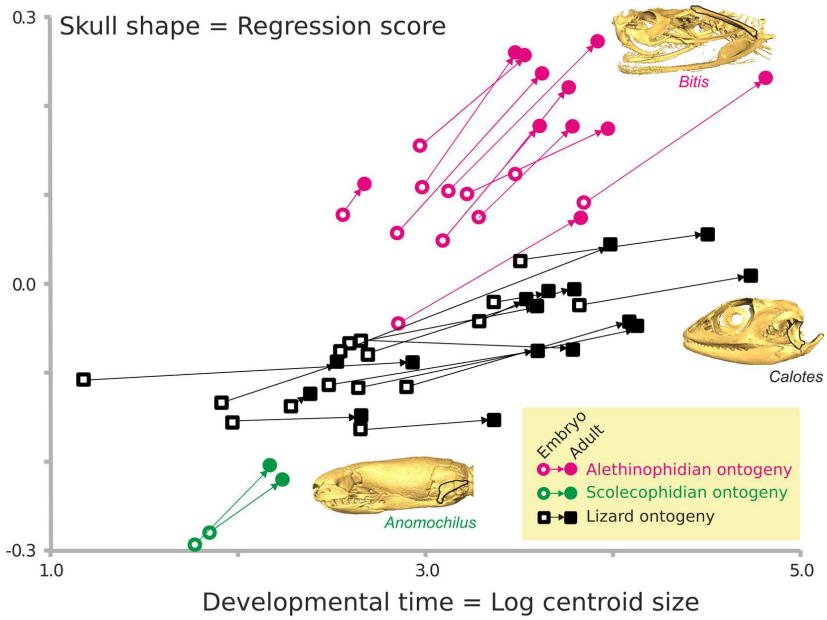

Figure 13 Evolutionary allometry, ontogenetic trajectories, and heterochrony. The Regression analysis of 2D skull shapes (regression score) onto developmental time (log-centroid size) for lizards (black squares), scolecophidian (green circles), and alethinophidian (pink circles) (Publication I). Embryos are shown as open circles (for snakes) or squares (for lizards). Arrows indicate the direction of skull shape development. Note the steeper trajectories of snakes, particularly expressive among Alethinophidia. This was indicative of peramorphosis through acceleration. Lateral views of 3D-rendered skulls show the difference of the quadrate bone shape (outlined in black), an important marker of the level of skull shape development.

I investigated the earliest and the latest limits of head/skull embryonic development as they are more comparable at a macroevolutionary scale. First, I compared the offset of ossification of both frontal and parietal bones at the latest embryonic stage (pre-hatchling or stage 10 as defined for *Boaedon fuliginosus*). Advanced ossification was consistently found in Alethinophidians than in most lizards and scolecophidians (Figure 14).

Finally, a comparative analysis of five head traits coded for embryos at 0-1 days post-oviposition (dpo) further confirmed that snakes have already a more-developed external skull phenotype than lizards (II) (Figure 14). *Highlighting, these comparisons between lizards and snakes were first done in studies (I) and (II).*

Summing up, heterochrony by the process of peramorphosis through the mechanism of acceleration of the skull shape development is most likely underlying the overall skull shape innovation seen in alethinophidian snakes and the radiation of Caenophidian snakes.

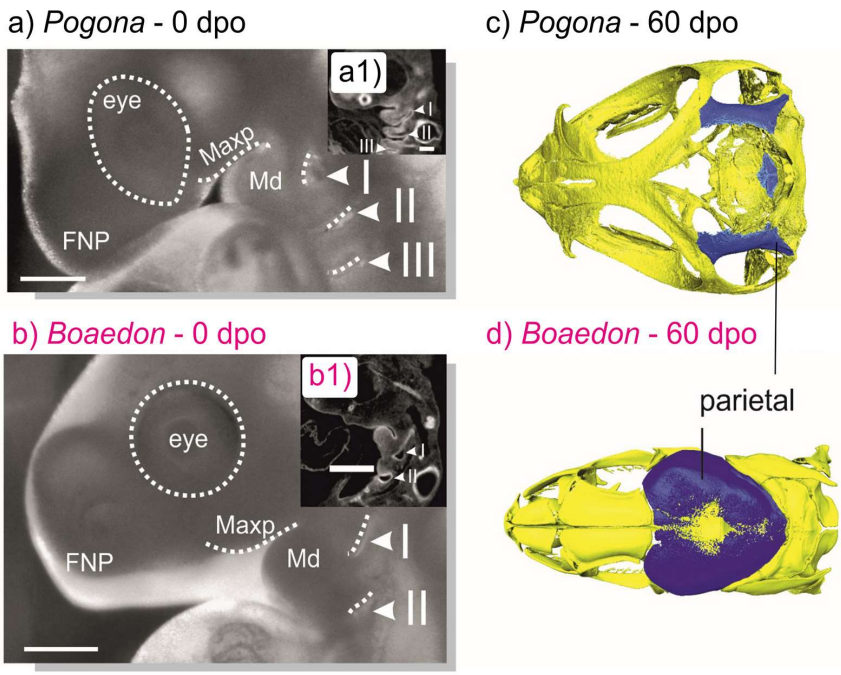

Figure 14 Comparison between embryonic traits between a lizard (*Pogona vitticeps*) and an Alethinophidian snake (*Boaedon fuliginosus*) at the same embryonic stage for two species with an equal number of days post-oviposition (dpo) (Publications I and II). a) and b): the lateral view of the head of the snake embryo shows two pharyngeal slits (I and II) while three are observed in the lizard embryo (I, II, and III). a1) and a2) show a cross-section of the PTA-stained head of embryos, confirming the pattern of 3 to 2 pharyngeal slits. The snake eye, Frontonasal prominence (FNP), Maxillary prominence (Maxp), and Mandible (Md) are more developed in snakes. c) and d): the parietal bone is considerably more ossified in the snake embryo than in the lizard embryo. Thus, the snake skull/head development proceeds faster than in lizards.

5) DISCUSSION

5.1) THE RELATIONSHIP BETWEEN SKULL FORM AND FUNCTION

Gould & Lewontin (1979) wrote:

> Too often, the adaptationist programme gave us an evolutionary biology of parts and genes, but not of organisms. It assumed that all transitions could occur step by step and underrated the importance of integrated developmental blocks and pervasive constraints of history and architecture. A pluralist view could put organisms, with all their recalcitrant, yet intelligible, complexity, back into evolutionary theory. (p. 163).

I embraced the scientific spirit described above, expanding the discussion of the origin of snakes from coded characters for phylogenies to shape of the skull as a whole regarding its development, phylogeny, and ecology all integrated to understand the evolutionary history of snakes. The snake skull morphologies are diverse and complex (Cundall & Irish, 2008). Initially, it was not clear to me, based on previous studies, if there was a strong relationship between skull shapes and habitats. Most focus had been given to the relationship between skull morphology and diet because snakes are gape-limited (Moon et al., 2019). However, the skull is a composite structure with a diverse range of bone sizes and shapes, complex developmental basis, and executes potentially competing functional roles beyond diet, including locomotion, protection, sensing, among others. It is truly a challenging model for evolutionary studies (Hallgrímsson et al., 2009).

Thus, it was a brave scientific move to attempt to detangle neutral evolution, phylogenetic structure, developmental processes, and ecological associations in the skull of snakes in comparison to lizards in terms of use and locomotion in different habitats. Making it more challenging, the literature in the field of snake and lizard skull evolution has a long history, which is vast and patchy (Cundall & Irish, 2008). Throughout the research, it became evident that that field was lacking a unifying understanding that would connect anatomy and phylogeny with functional morphology and development. Furthermore, the problem of the snake origin, with its intricacy and overturns, had been mostly investigated considering discrete adult body features. Only recently has more skull fossils become available, but they had never been compared in quantitative terms. If we are talking about the early ecology of snakes, we also need to understand the relationship between form and function, so a quantitative type of

investigation. Geometric morphometrics of the skull shape turned out to be an informative method to shed new light on the patterns of early skull evolution and ecologies of snakes, but it is subjective to statistical approaches and so should continuously expanded and challenged. Finally, this dissertation and included publications is the fruit of embracing complexity with curiosity and determination.

In terms of the morphospace occupation, the separation between lizards and snakes, as well as the unification of convergent fossorial lineages, has also been recently supported by another large-scale study with a semi-landmark/landmark approach to quantify skull shape (Watanabe et al., 2019) and also by traditional morphological approaches (Simões et al., 2020).

Furthermore, the significant association between skull shape and ecology, or form and function, are compatible with most previous studies that looked at lizards and snakes individually (see introduction). Uniquely, it was shown in this dissertation and included publications that the association between skull shape and habitat is a widespread phenomenon among squamates. This new notion has been also supported by recent geometric morphometrics studies of lizards and/or snakes (Watanabe et al., 2019; Gray et al., 2019a; Gray et al., 2019b). Additionally, previous studies have shown that the geometric skull shapes of lizards and snakes are also correlated with diet (Monteiro & Abe, 1997; Stayton, 2005, 2006; Daza et al., 2009; Sagonas et al., 2014; Klaczko et al., 2016; Fabre et al., 2016; Dollion et al., 2017; dos Santos et al., 2017; Watanabe et al., 2019; Sherratt et al., 2019b).

The identification of adaptations relies heavily on the comparative method, which in essence searches for correlated evolution between characters and environments (Harvey & Pagel, 1991). This is challenging because correlation does not imply causation, as functional studies need to be performed. For Squamates, previous functional studies showed the importance of habitat in the skull shape evolution of lizards (Barros et al., 2011) and diet for snakes (Forsman, 1996; Moon et al., 2019). One could expect that different functions would be anatomically partitioned in the skull, so that the signal would be specific for some ecology in one part of it whereas for another in other parts. Modularity would be expected. However, the skull of squamates was found integrated at a macroevolutionary level (Watanabe et al., 2019).

In biology, integration refers to the tendency for the developmental system to produce covariation at the phenotypic level or, in other words, we find a correlation between traits (Hallgrímsson et al., 2009; Cheverud, 1982; Pigliucci, 2003). The analysis of morphological integration was first formalized by Olson and Miller (1958). They stated that different levels of integration would be expressed by different degrees of correlation among measurable characters. Integrated parts would then evolve along the same evolutionary trajectory. Fitting to that expectation, in the

phylomorphospace estimated here, a directional trend is seen for most alethinophidian lineages and fossils between values of (-) PC1 and (+) PC2, except for the repeated evolution of fossorial lineages.

It is plausible that functions such as locomotion, sensing, defense, and feeding are not necessarily strongly competing with one another so that one overwrites the others in terms of morphological signature in certain regions of the skull. The skull is multifunctional. In an integrated skull, multiple ecological functions are co-shaping morphology, perhaps indicating that emergent trade-offs are in balance and that they might be minimized between functions. Development can limit evolution to reach a maximum fitness when one looks solely at the phenotype (Salazar-Ciudad & Marín-Riera, 2013), but when looking at its function, maximum fitness can be achieved by the many-to-one relationship of phenotype to function (Polly, 2013; Salazar-Ciudad & Marín-Riera, 2013). Moreover, a recent study has shown that trade-offs might instead benefit evolutionary transitions instead of constraining them (Polly, 2020). Functional trade-offs could carry evolution across lower performance valleys, as maximum performance for a specific demand might not be achieved, but they may represent the optimal solution in any environment in which an organism experiences competing for functional demands (Polly, 2020). An integrated skull, in this regard, maintains a shape balance even when strong selection would be expected to favor certain shapes linked mostly with a specific functional demand. An exception to this reasoning might be fossoriality, which leads to dramatic functional changes in all other aspects of ecology in favor of habitat locomotion. This must be further studied.

On the other hand, it is well-known that modularity is a widespread phenomenon in biology that has been associated with evolutionary diversification, novelty, and disparity between body parts (Wagner, 2007). Modules are expected to represent evolutionary building blocks, and so evolution could generate different outcomes between them. In other words, modularity refers to the partitioning of variation in a structure among correlated traits in association to function, phylogeny, development, and genetic networks (Wagner, 2007; Klingenberg, 2013b). For instance, think of the forelimbs and hindlimbs in snakes, which have long lost their forelimbs but hindlimbs persisted in some extant and extinct lineages (or they might as well have reversed) (Leal & Cohn, 2016). In extant snakes, the vestigial hindlimbs were co-opted as clasps that aid mating and in the formation of the hemipenis - an exaptation (Tschopp et al., 2014).

Modularity has also been investigated regarding the skull shape of lizards and snakes. Monteiro and Abe (1997) identified modularity in the skull of the lizard *Tupinambis merianae* with a clear interplay between ecology (dietary shifts) and bone development. Sanger et al. (2012, 2013) found that skull modularity is not conserved among lineages of *Anolis* lizards, for instance, *A. sagrei* and *A. cybotes* have convergent integrated skulls, whereas the long-faced species - *A. carolinensis* and *A.*

bahorucoensis - have both evolved a novel, rostrum-specific module. Lazić et al. (2015) identified modularity in *Podarcis muralis* associated with developmental modules based on the investigation of the offset of skull ossification (anterior bones ossify faster than posterior ones). Powell et al. (2017) interpreted modularity in the dermatocranium of *Phrynosoma hernandesi*. Conversely, Taylor et al. (2017) did not find modularity in the skull of casque-headed lizards by comparing the crest against the rest of the skull, but they did not find significant integration in males either, only among females. Urošević et al. (2019) identified different levels of modularity by analyzing 14 species and 8 genera of Lacertidae lizards. At the static level, they found separation between anterior and posterior functional regions of the skull. At the ontogenetic and evolutionary levels, modularity was detected for the ventral cranium. Andjelković et al. (2017) found that there is a high level of morphological integration between the braincase and elements of the feeding apparatus in snakes of the genus *Natrix*, but the snout elements (premaxilla and nasals) express the lowest degree of correlation with the rest of the skull. This is compatible with their delayed onset of ossification (Werneburg & Sánchez-Villagra, 2015). Another exception to the integration pattern in squamates, even at the macroevolutionary scale, might be the shape of the quadrate (section 5.3).

From these studies, it seems that modularity can be present within and among closely related species, but that pattern might be obscured or less common across distantly related lineages. Modularity within lineages is highly variable due to differences in the way covariation is partitioned. Comparing all lineages might obscure nuanced patterns of modularity in favor of integration. Conversely, skull integration might indeed be more common in a large scale, so largely overwriting the signal of the few lineages that have modular skulls. Modularity in lower levels might not be expressed in the adult phenotype.

5.2) THE ECOLOGICAL ORIGIN OF SNAKES

“The origin of snakes has recently become a paleontological 'hot potato'” (Rieppel & Kearney, 2001, p. 110). This research program provided a new and integrative ecological hypothesis as well as an evolutionary scenario for the origin and radiation of snakes. A terrestrial early-ancestor of snakes, meaning a population of lizards that likely inhabited Gondwana in the Jurassic, was most-likely the precourse of the crown-snake lineage of fossorial snakes in the Lower Cretaceous. This unites the hypotheses of a terrestrial ancestor with that of a fossorial ancestor, while rejecting the marine hypothesis. The exclusion of a marine origin has also been supported by a complementary approach to study skull shape and rates of evolution (Watanabe et al., 2019) and nanometric diversity of scales (Arrigo et al., 2019). I suggest that future studies direct attention to understanding in more detail the likely terrestrial/fossorial ecology of the early snake

ancestor.

A terrestrial to fossorial change in the ecology of early snake ancestors had not been previously presented. This is surprising given that this same ecological change was recovered as the most likely scenario in the evolution of other elongated, limb-reduced lizards such as amphisbaenians (Müller et al., 2011) and Gymnophthalmidae (Barros et al., 2011). Likely, this proposition was mostly delayed for snakes because most previous studies were focused on the evaluation of the phylogenetic position of fossils and did not pay the necessary attention to the tree nodes that connect stem lineages. The conceptual shift of looking at the evolutionary change between internodes allowed us to evaluate the mode and time of the ecological transitions in the early snakes in a unique fashion. This is dramatically different from looking for the sister-group lineage of snakes, which offered little resolution and is, at least partially, based on misconceptions of morphological evolution.

Furthermore, a terrestrial to fossorial transition is more likely based also on independent studies. Fossoriality has evolved multiple times in lizards and snakes, and in most cases, from a four-legged, terrestrial ancestor, so it is an expected evolutionary path (Wiens & Slingluff, 2001; Wiens et al., 2006). Importantly, a burrowing lifestyle is not necessarily correlated with total limb loss but is with elongated bodies (Wiens & Slingluff, 2001).

The proposed ecological scenario also seemly rejects the hypotheses of head-first (Caldwell et al., 2015) and body-first evolution (Longrich et al., 2012). The view supported here is of an integrated evolution of the snake *bauplan*, although limbs follow a more specific trajectory. This is treated in terms of developmental processes and its mechanisms in the section 5.3. However, further studies should be pursued to integrate the skull and body shapes with geometric morphometrics.

A fossorial ecology for crown snakes is also compatible with most-recent scientific findings based on the fossil record and ancestral-state estimations (Apesteguía & Zaher, 2006; Wiens et al., 2006; Cundall & Irish, 2008; Brandley et al., 2008; Longrich et al., 2012; Martill et al., 2015; but for an opposite view, see the review by Caldwell et al., 2019). Furthermore, terrestriality for the early ancestor of stem snakes fits well with late 19th century and early 20th century hypotheses, although they lacked phylogenetic and comparative methods for formal evaluation (Cope, 1869; Janensch, 1906; Camp, 1923). Finally, Vidal and Hedges (2004) discussed that an evolutionary change of a specialized marine group into a terrestrial one has never been documented, not at least since the origin of tetrapods (Dickson et al., 2020).

Why does the marine hypothesis remain as an acceptable alternative in contemporary discussions of the early ecology of snakes in light of a large and variable number of contrary evidence to that hypothesis? I believe that is still the case because no other study before the ones included and further

presented in this dissertation had approached the problem of the origin of snakes with an integrative experimental design. This time around, statements like "but you did not look at this type of data" or "but you did not include this important taxon" are not valid. By combining a variety of large-scale sources of data (extant and fossil, adult and embryos, phylogeny and ecology, mathematical and statistical approaches), a terrestrial-to-fossorial change in the early evolution of snakes is strongly and convergently supported. Additionally, several researchers were looking at the problem not only with restricted data (morphological traits and phylogeny) but also with a linear, static, and, at times, misleading conceptual framework of seeking only to recover the sister-group/taxon to be “able” to deduce the ancestral ecology. Evolution is instead mostly dynamic, and one must focus attention at evolutionary differences between ancestral nodes, which can be holistically estimated. Looking at the phylogenetic position of single fossils is prompt to generate confusion. A few fossils are limited in describing with confidence the shape and size of ancestors. Fossils are tip specimens on their rights and might not represent ancestral traits or populations (Crisp & Cook, 2005). Fossil lineages also undergo evolutionary changes since their common ancestor split with living lineages (Prothero & Buell, 2007). They might have a combination of traits that were present in the ancestor too, but that cannot be granted from deduction and so that approach has reduced scientific value. Fossils can confirm that certain traits and groups were already present at certain geological periods, but they cannot be necessarily equated to ancestors or be seen as “missing links” (Prothero & Buell, 2007).

More interestingly, fossils that were not included in the analyses, are an excellent material to contrast with ancestral estimations. The crown-snake ancestor has likely evolved in the Lower Cretaceous. However, no other snake with a preserved skull than *Tetrapodophis* (Martill et al., 2015), which has its taxonomical status under disputes (Lee et al., 2016), has been found that is dated from the Lower Cretaceous. On the other end, Jurassic fossils are represented by skull fragments (Caldwell et al., 2015). The discovery of new snake skulls from the Lower Cretaceous and better-preserved ones in the Jurassic will allow for comparisons with estimated ancestors. Additionally, a recent study has grouped several fossil snakes in a parallel radiation to the alethinophidian clade, but this hypothesis has not yet been evaluated in the context of skull shape evolution (Garberolio et al., 2019a).

The crown snake ancestor was also estimated to be a small fossorial, but not as miniaturized as seen among extant blind snakes. These findings match well with the small skull size and fossorial ecology of early-branching lineages represented by the Upper Cretaceous *Coniophis* (Longrich et al., 2012), Upper Cretaceous *Najash* (Apesteguía & Zaher, 2006; Garberoglio et al., 2019a), and with the Lower Cretaceous *Tetrapodophis* (Martill et al., 2015). In functional terms, small head size is already compatible with the

force requirements for a head-first burrowing behavior (Laduke et al., 2010 and references therein). A long body (2 m) might be seen as incompatible with fossorial behavior as seen in *Coniophis* (Longrich et al., 2012; Palci et al., 2018), but even long and large snakes can still burrow (Bruton, 2013). Interestingly, a new blind snake fossil (*Boipeba tayasuensis*) from the Upper Cretaceous supports our size-evolution model of increased miniaturization among blind snakes (Fachini et al., 2020). Miniaturization within the scolecophidian lineage had been also suggested before (Kley, 2006; Kley & Brainerd, 1999). The findings described here oppose the ongoing discussion of large versus miniaturized snake ancestors (Scanlon & Lee, 2000; Scanlon, 2006; Hsiang et al., 2015; Caldwell, 2019), reframing it as a smaller ancestor than the one of alethinophidian snakes and larger than the one of scolecophidian snakes. Of course, fossil comparisons are contentious because one would have to assume that those fossils are representative of the early size of their most-recent common ancestor (poor species clades offer little information on that).

Finally, the evolutionary change in size was correlated with changes in shape, particularly in the early evolution of snakes. Allometry might have facilitated the evolution of a fossorial behavior in a microevolutionary scale. However, allometry is not a requirement for the evolution of fossorial forms in lizards, for example, amphisbaenians. The association between allometry and the origin of fossoriality will require more investigations.

5.3) THE MORPHOLOGICAL AND ECOLOGICAL RADIATION OF SNAKES

“It is useful to regard evolution as the control of development by ecology (...) The emphasis is therefore on the phenotype, on the level where adaptation occurs. And the phenotype is development." (Van Valen, 1974, p. 115). This dissertation and accompanying publications showed that the snake skull develops faster than those of lizards. Fast development would have generated novel skull shapes and functions through peramorphosis in balance with natural selection.

Evolutionary novelty can be simply defined as any biological structure that creates conditions for an organism to exert new functions that were not present in its ancestor (Mayr, 1960) or “Novelty reflects the formation of newly individuated characters, features of the organism which were not present in ancestral species” (Erwin, 2017, p. 2).

Novelties can be divided into three morphological categories: new body plans, new structures with no homology within deep ancestors, and structures that have undergone substantial departure from the ancestor condition (Müller, 2010). Furthermore, Hallgrímson et al., (2012) proposed that novelty is associated with changes in the evolutionary trajectories of organisms in close association to fitness landscapes. Gould (1977) and Alberch et al. (1979) instead associated novelty with changes in the

ontogenetic trajectories of descendant lineages in comparisons to ancestors by heterochrony in association with natural selection. They described evolutionary novelty as a result of prolonged developmental time (hypermorphosis) or then increased rates of development (acceleration). The two former definitions are focused on traits, so prone to subjectivity, and anything could be potentially considered novelty (Pigliucci, 2008). The last two definition are focused on processes and based on a formal analytical treatment that have expected patterns, thus, more ideal. Alberch ´s approach also does not neglect development and see it as a crucial step between the genotype and the phenotype. As Alberch (1980) stated "In evolution, selection may decide the winner of a given game but development non-randomly defines the players" (p. 665).

The observed unique morphospace occupation of alethinophidian snakes fit well to notion of novelty. It is interesting to notice that the same habitats in lizards are linked with different skull morphologies in snakes, but for fossorial lineages, which are mostly convergent. I hypothesize that snakes broke evolutionary and developmental constraints (Maynard Smith et al., 1985; Alberch, 1989) that "kept" fossoriality in a specialized "dead-end". From a fossorial ancestor, snakes radiated into a variety of ecologies.

Gould (1977) linked heterochrony with ecological strategies. Snakes evolved from lizards, and so we can assume that today´s ontogenetic trajectories observed among lizards are also representative of their ancestral ones. To improve the strength of that assumption, ontogenetic trajectories from all major lineages of lizards were included. The pattern of morphospace occupation by snakes signaled that it could be an evolutionary outcome of peramorphosis (Gould, 1977; Alberch et al., 1979). However, the distinction between its mechanisms (hypermorphosis or acceleration) is not possible from the morphospace. The regression approach showed that acceleration in skull development was most likely the mechanism in place. Interestingly, Watanabe et al. (2019) also found snakes, iguanians with cranial ornamentation, and fossorial lizards show the highest rates of skull evolution. Simões et al. (2020) reinforced that the origin of snakes is marked by the highest rates of phenotypic evolution in diapsid history, which is largely explained by skull features.

To explain the radiation of snake skulls from a fossorial ancestor, I presented here (in this dissertation, not in the publications) an evolutionary model that is based on a feedback-loop mechanism between ecology (natural selection) and ontogeny (rates of development). The evolution of snake skulls would represent an example of the balance between ecology and development considering what the theories that have been laid down by Van Valen (1974) and Gould (1977). In accord with my new model, complementary to descriptions presented in the publications, ontogenetic changes in the skull shape would have been reinforced by selective pressures linked with habitat, but, most importantly, diet. The presence of larger prey in a new geographic region could be exploited by snakes with a

larger gape size. In a feedback response, new phenotypes would have emerged by increased rates of skull development affecting skull shape that was likely linked with the rise of wider gapes (see discussion on the quadrate bone below). Some of these new phenotypes would have allowed snakes to ingest even larger prey. Ultimately, this dietary shift would have fostered radiation (Cundall, 1995; Greene, 1983; Cundall & Greene, 2000).

The evolution of gape size is a disputed topic. Gape size might have increased one or multiple times from limited-gape ancestors in snakes (e.g., Cundall & Irish, 2008; Pyron et al., 2013; Harrington & Reeder, 2007; Miralles et al., 2018; Burbrink et al., 2020) or from an ancestor that already had a large gape size (Rage & Escuillié, 2000; Caldwell et al., 2007; Scanferla & Bhullar, 2014; Hsiang et al., 2015; Garberoglio et al., 2019b; Strong et al., 2020). Fortunately, the debate can be simplified as of distinct claims about the level of macrostomy, which has increased within several lineages of alethinophidian snakes (Harrington & Reeder, 2007; Miralles et al., 2018; Moon et al., 2019). Reversals also took place, so bear in mind that my complementary skull evolution model does not aim to be representative of linear evolution. My skull evolution model presented firstly here fits well with a scenario of increased macrostomy. Moreover, our snake ancestral estimation, as described in the publication I, supports an early ancestral gape-size (Figure 4b, Publication I) - if one considers that gape-size can be estimated from the shape and rotation of the quadrate-bone (Scanferla, 2016; Moon et al., 2019).

The prenatal ontogenetic changes in the quadrate bone had major relevance to establish the observed evolutionary patterns in the adult morphospace and ontogenetic morphospace linked with macrostomy. The anteroposterior elongation of the quadrate bone and the antero-posterior projection of its ventral articular surface (towards the body) throughout ontogeny is compatible with other sources of quantification such as linear metrics and angles (Scanferla, 2016; Silva et al., 2018). These observations are also compatible with earlier embryonic studies based on a few samples that had shown that the snake's quadrate changes from an anteroventral inclined position in early embryos, passing through a vertical position and, finally, a posteroventral inclined orientation in late embryos (Kamal, 1996). This quadrate rotation is paralleled by the elongation of the supratemporal throughout development (Peyer, 1912). The description of embryos of the snake *Thamnophis radix* has recently also reinforced the importance of ontogenetic changes in the morphology of the quadrate bone for the adult morphology (Strong et al., 2019). Moreover, postnatal skull shape changes, particularly in the quadrate, have been shown to parallel increased gape size and dietary shifts in the evolution of snakes (Scanferla, 2016; Silva et al., 2018). Diet would have been a major selective force that led to directional increase in the rate of skull shape development. In turn, it led to skulls that could ingest larger prey. Conversely, fossorial habitat opposes that trend.

Unfortunately, the underlying molecular mechanisms leading to observed shape changes in the quadrate of snakes are unknown. It is known that, at the molecular level, rapid genomic reorganizations were observed in snakes (Di-Poï et al., 2009; Castoe et al., 2014), but their relevance for the evolutionary model described remains elusive. Molecular mechanisms of quadrate development are better known for mammals, but it is there highly modified into an ossicle of the middle ear (Tucker, 2017), thus bearing limited information for snakes. On the other hand, the shape changes observed here for the quadrate can be explained by the developmental mechanism of acceleration in the rate of skull development. The fossil record corroborates the presented model. Scanferla and Bhullar et al. (2014), by comparing the postnatal ontogeny of the fossil snake *Dinilysia* with published information for lizards and snakes, found a higher level of skull ossification and great posterior elongation of the supratemporal. They suggested, "that accelerated maturity of the head skeleton since perinatal stages is an apomorphic trend shared by *Dinilysia* and extant snakes" (p. 566).

The labile response of skull rate development to new environments and diets was likely paramount in the early phases of occupation of new regions. Phenotypic plasticity is also common in snake skulls (Moon et al., 2019). Thus, the great departure of snake skull shapes from their ancestral condition is seen here to have allowed for new functions to emerge like preying on large mammals and birds. This would have fostered species and ecological radiation among boas, pythons, and, most remarkably, caenophidian snakes (Cundall, 1995; Greene, 1983; Cundall & Greene, 2000).

In support, a model of non-random speciation–extinction process found that the alethinophidians underwent species radiation (Ricklefs et al., 2007). Of note, patterns of species richness and radiations were also found to be influenced by mass extinction events, and snakes would have taken a long time to recover their diversity from such catastrophic moments (Longrich et al., 2012; Hsiang et al., 2015). This tells us that evolution is a combination of intrinsic and extrinsic factors. Mass extinctions remain to be combined with skull shape evolution in squamates.

On the other hand, scolecophidians show a different pattern. They have shorter ontogenetic trajectories in the same range as those of fossorial lizards, slower ossification rates, and head development like lizards. Most lizards, differently from alethinophidian snakes, remain with mostly unossified skull rooftops at the time of hatching. Completion of skull ossification in lizards takes place postnatally (Maisano, 2001). Similarities between scolecophidians and lizards could represent either ancestral-state conservation or then paedomorphosis. Paedomorphosis had been previously suggested (Kley, 2006; Kley & Brainerd, 1999; Palci et al., 2016), but never formally tested until this dissertation. The observed trend of reduction in size and the fossil record (Fachini et al., 2020) also support the

hypothesis of paedomorphosis as presented here.

On the other hand, mosaic or dissociated heterochrony is also a possibility for the skull of snakes, for example, paedomorphosis linked with dissociation of snout bones that generated more mobility has been proposed based on comparisons of the onset of ossification and ancestral-state estimation of ontogenetic series (Werneburg & Sánchez-Villagra, 2015). Such dissociation of bones has been hypothesized to play an important role in the evolution of feeding in snakes (Irish, 1989). Heterochrony patterns also were found to vary depending on the taxonomic level supported by formalized mathematical approaches. This is expected because comparisons are between descendants and ancestral states (Sherratt et al., 2019b; Gray et al., 2019b).

Recently, Simões et al. (2020) discussed that "we predict that future findings will soon be able to reveal fundamental clues on the potential factors enabling phenotypic innovation decoupled from taxonomic radiation during early snake evolution." (p. 8). However, one must reaffirm that the early fossil record is poor for snakes. It remains unclear if snakes had already ecologically radiated early on in their evolution between the Jurassic and the Lower Cretaceous. Snakes had already radiated in the Upper Cretaceous (Longrich et al., 2012). The available fossils were interpreted to reflect variable ecologies in the Jurassic (Caldwell et al., 2015), but that remains elusive before the discovery of better-preserved specimens.

From the phylomorphospace recovered here, the first phase of the evolution of snakes was most likely conservative in terms of morphological, taxonomical, and ecological diversification. It is more likely that snakes at this early phase diversified in skull shapes mostly linked with different requirements for terrestrial life with fossorial behavior. Morphological, taxonomical, and ecological radiation would only take place much later in the evolution of the group: first in the Upper Cretaceous and again after the Cretaceous mass extinction event. These reasoning is compatible with a proposition that early adaptive radiations are less common than had been anticipated (Harmon et al., 2010), meaning that early bursts of species followed by stasis would be rare. Instead, selection (fitness peaks) and developmental constraints would be relevant throughout the diversification of a clade by directing morphological changes. In accord with my skull evolution model, as development and selection began to interact more closely through a feedback-loop mechanism, tightly regulated by alterations in the rate of development, snakes have found the evolutionary conditions to radiate (boas, pythons, and caenophidians). Early radiation in the Jurassic, if confirmed, will be likely explained by other processes than those in my skull model.

On the other hand, the combined phylogeny of Garberoblio et al. (2019a) and discussions by Caldwell et al. (2015) have suggested the snake had much earlier morphological and ecological radiations. Garberoglio et

al. (2019a) are vague about the ancestral snake ecology. *Najash* was maybe a terrestrial, fossorial, or even a semi-fossorial organism; or just because the topology presented by Garberoglio et al. (2019a) seems to reinforce the notion of an early ecological radiation for snakes. Interestingly, the phylogeny described in Garberoglio et al. (2019a) leans towards supporting Caldwell et al., (2015)'s views of an early ecological radiation for snakes but less so Garberoglio et al., (2019b). One could be led to interpret that if the Cretaceous and other relevant fossils are together in a sister group and represent multiple ecologies then the ancestral ecology of snakes would be harder to know because all those ecologies are equally "sister ecologies" and so "ancestral ecologies". That is a limited assumption, one or multiple fossils as the sister lineage is not enough to state the ancestral ecology of snakes. This is discussed in the next section as the "sister-group fallacy". If we continue to state the snake ecology based on this conceptual framework, confusions will just increase, and we will be walking in circles where new data just leads us to assimilation not accommodation of ideas.

The alternative that was presented elsewhere (Caldwell, 2019), which is hardly testable, is that the snake ancestor was then an intermediate and so a complex chimera in morphological and ecological terms - a semi terrestrial organism that swam in saline waters near mangroves and that preyed on big animals while climbing on mangrove trees with its four tiny legs (as illustrated in the cover of Caldwell (2019)'s book). Again, further analysis with complementary methods should be performed to confirm this hypothesis.

Of final note, the ecological and morphological radiation of snakes is most likely also linked with body features, which were not studied in this doctoral project. An integration of body and skull shape remains to be done in future studies. Of note, the elongated body of snakes has been shown to have evolved by the acceleration of the rate of somitogenesis (Gomez et al., 2008), leading to a larger counting of vertebrae than in lizards (Müller et al., 2010). Seemingly, the head and body of snakes evolved in synergy, rejecting a head-first hypothesis (Caldwell et al., 2015) and a body-first hypothesis (Longrich et al., 2012). This is also compatible with previous findings based on linear morphometrics of the body shape that showed that the selective pressure of digging under soil deeply influences the morphology of lizards and reached extremes among snakes (Wiens et al., 2006).

5.4) THE ECOLOGY OF A SNAKE ANCESTOR: A CONCEPTUAL ISSUE?

On the ecological origin of snakes, the prominent evolutionary biologist Olivier Rieppel (1988) wrote:

> Although the riddle appears to be rather easily solved if looked at from a

> somewhat superficial perspective, it must be admitted that an acceptable solution is no closer than at the beginning of this century, and while faith in the promise for progress of a purely observational approach vanishes, conceptual issues are more and more thrown into focus. (p. 37).

Our data and understanding about the ecological origin of snakes improved. This dissertation and its included publications are thought to have largely contributed. Yet, conceptual issues do persist.

The morphological, ecological, and developmental scenario for the origin of snakes presented in this dissertation has been well-received by independent studies with complementary approaches (e.g., Roscito et al., 2018; Miralles et al., 2018; Broeckhoven & DuPlessis, 2018; Palci & Lee, 2018; Tamagnini et al., 2018; Watanabe et al., 2019; Bardua et al., 2019; Eymann et al., 2019; Moon et al., 2019; Sherratt et al., 2019b; Macrì et al., 2019; Čerňanský & Stanley, 2019; Camacho et al., 2019; Arrigo et al., 2019; Souto et al., 2019; Macaluso et al., 2019; Feijó et al., 2020; Bon et al., 2020; Strong et al., 2020; Nicula, 2020; Khannoon et al., 2020; Inoue & Saitou, 2020; etc).

In a review on feeding evolution in snakes, Moon et al. (2019) wrote:

> In a recent integrative study of skull evolution in snakes, Da Silva et al., (2018) concluded that all snakes and their sister group evolved from a surface-dwelling terrestrial ancestor with non-fossorial behavior. Yet the Crown-snake ancestor had a skull shape adapted for fossoriality. Although the debate on the origin of snakes is far from resolved, a consensus is now emerging that the earliest snakes, or at least the stem snakes leading to the crown group of snakes, were fossorial or semi-fossorial animals that had a functional intramandibular joint and ate relatively large prey. (p. 528).

One author, who resurfaced the marine origin of snakes, criticized the studies included in this dissertation as having a mythical foundation, a teleological mindset, and would have used pseudo-citations to back up claims (Caldwell, 2019). By constructing a heated and personal argumentation, Caldwell (2019) contradicts our current appreciation of processes linked with the growth of the scientific thought (Kuhn, 1970; Mayr, 1982).

Before addressing his criticism, I presented three general conceptual issues in the field of snake evolution that need to be addressed before it and will help in the rebuttal: misunderstanding of tree topologies and deduction of ancestors based on single fossils (according to Krell & Cranston, 2004; Baum et al., 2005; Crisp & Cook, 2005; Omland, 2008), as well as *ad hoc* hypotheses that are unfalsifiable (according to Popper, 1959).

Several studies about the ancestral ecology of snakes have incorrectly interpreted outgroups by directly equating them to the ancestral morphology and ecology of ingroups (Crisp & Cook, 2005). This is not

trivial. Misinterpretations of phylogenies have long been debated because they produce misleading ecological and evolutionary scenarios (Krell & Cranston, 2004; Baum et al., 2005; Crisp & Cook, 2005; Omland, 2008).

Publications with that issue will describe morphological transitions as sequences of tip specimens from the top-left to the top-right of topologies. That is conceptually rooted, even if implicitly and unaware, in the "ladder of progress", in this case reframed to a horizontal axis instead of a vertical line as seen in pre-revolutionary times (Omland, 2008). This way, phylogenetic trees ended up being an image to fit minimal evolutionary standards but does not fully reframe the conceptual understanding about morphological evolution.

Studies with that conceptual issue will describe species or clades as being "more basal" (or "primitive") to the "more derived" (or "advanced") ones. Phylogenetic trees are not meant to be described that way (Krell & Cranston, 2004; Omland, 2008). The relative ordering of tree branches is not the focus but the ancestral nodes (Figure 15).

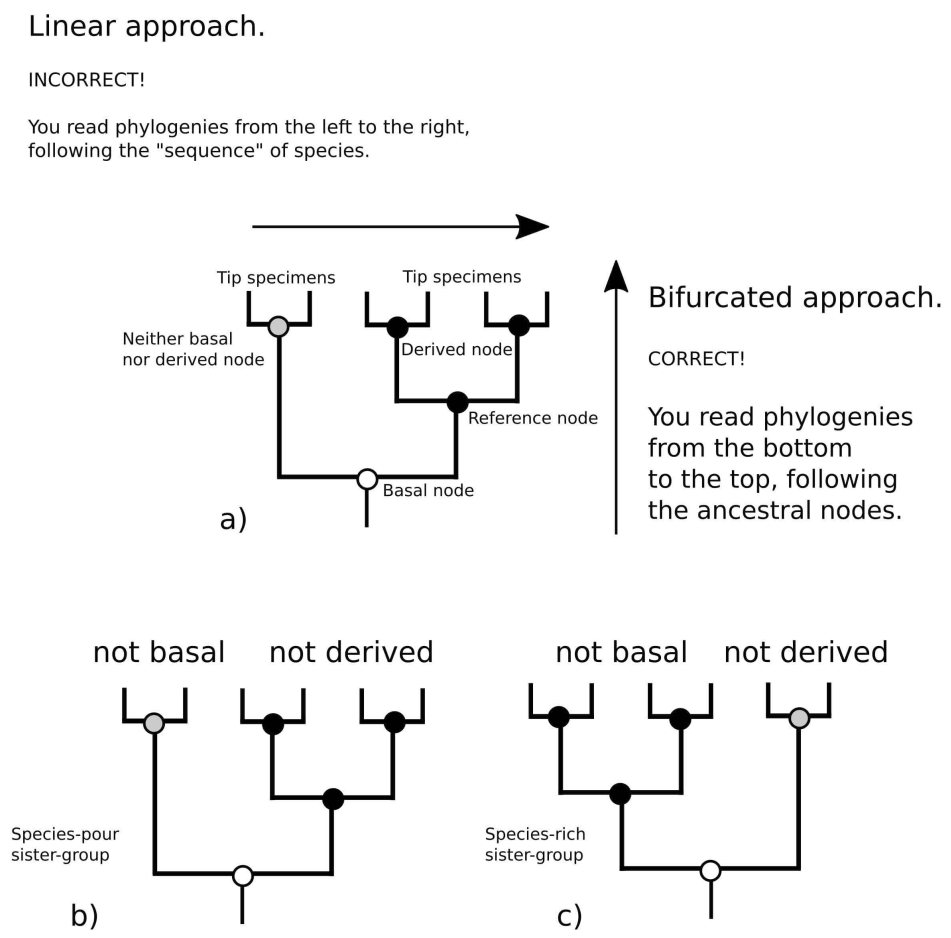

Figure 15 Two approaches to reading phylogenies: linear versus bifurcated. a) shows a hypothetical topology where nodes are differentially colored. Specimens of any clade are neither primitive nor derived from each other, only is valid for adjacent ancestral nodes. b) and c) show the same topology but rotated concerning each other. b) has the species-poor group on the left-side and c) on the right side. This change does not alter evolutionary descriptions based on the bifurcated approach. Thus, basal and derived clades make no sense in phylogenies and evolution. It is a poor assumption to equate fossils with ancestors.

Misinterpretation of phylogenies in the field of squamate evolution is an old problem that has been resilient to conceptual change, just as expected from Conceptual Change Theory as described in section 1.1. of this dissertation (Posner et al., 1982; Duit et al., 2013).

Gauthier et al. (2012) provided clues about the root of those issues:

> Although the Cuvierian scheme of classification portrayed "affinities" in terms of a dichotomous hierarchy, the image of an underlying "series" of forms still dominated comparative biology well into the 19th century (Daudin 1926), as can be seen from Oppel's (1811) concerns about the continuity of the "lizard" with the snake "series." Louis Agassiz, in his famous Essay on Classification (1857), emphasized continuity of limb reduction in squamates, citing the graded series of intermediates between "lizards" and snakes as evidence for the completeness (plenitude) of the scheme of nature, which for him was ultimately rooted in Divine thought. Such views, of course, were famously opposed by Charles Darwin. (Gauthier et al., 2012, p. 4).

That rationale described above is deeply rooted in Aristotle's "Great Chain of Beings" and Linnaeus's *Systema Naturae*, with connotations of progress during change of life forms (Lovejoy, 1976).

Those misconceptions largely contributed to the scientific crisis in the field of snake early evolution, and, by extension, vertebrate evolution, with endless overturns and disputes on the early ecology of snakes that are largely the fruit of oversimplification.

Cladistics seems to be at the heart of the problem:

> provide support for a marine origin of snakes in the context of a cladistic analysis where character transformations can be followed through a cladogram (i.e., synapomorphies are organized in a hierarchical pattern from more inclusive to less inclusive clades; Lee 1998, 2005a, 2005b, 2009; Palci and Caldwell, 2010). (Palci et al., 2013, p. 1329).

That is permissive to old-fashion statements avoided in most fields of evolutionary biology such as the notion of primitive like "If these fossil snakes are to be considered primitive, (...)" (Palci et al., 2013, p. 1329).

This conceptual crisis stems from the so-called "sister-group fallacy" - a historical issue in biology that is fruit of misconceptions about the topology of phylogenetic trees. For example, the discovery of *Pachyophis* led to its interpretation as a transitional form and a marine origin of snakes.

That is a narrative co-opted and re-employed in the description/redescription of marine fossil snakes from the late 1990s and from thereon: “The recognition that the most primitive snake [*Pachyrhachis problematicus*] is marine does not support the fossorial hypothesis and suggests that an aquatic ancestry for snakes merits serious reconsideration." (Caldwell & Lee, 1997, p. 707).

Other clear examples follow below here:

> If the sister-group of snakes is a group of aquatic squamates that show a marked evolution towards highly modified limbs (Caldwell, 1996), then a reasonable alternative exists for the conventional view of a fossorial origin for snakes and snake characters. (...) This early snake shows numerous adaptations to marine environments (Lee & Caldwell, 1998) that could have been co-opted in later snake evolution for adaptations to terrestrial, and specifically, fossorial habits. (Caldwell, 1999, p. 139).

> Our conclusions, that the most primitive snake is the marine *Pachyrhachis*, and that the nearest relatives of snakes are the marine mosasauroids, are therefore unexpected. Neither *Pachyrhachis* nor mosasauroids could have been fossorial. At the very least, this means that fossoriality cannot be primitive in snakes, but rather must have evolved in snakes more derived than *Pachyrhachis*. It suggests that an aquatic origin for snakes, first proposed by Nopcsa (1908, 1923) but now largely ignored, merits serious consideration. (Scanlon, 1999, p. 146).

> Hypotheses regarding the adaptive origin of snakes, i.e., fossorial versus marine, must be made in the context of phylogenetic patterns that find a closest snake sister-group relationship with either an aquatic or a burrowing clade of squamates (see Caldwell 1999). Only then it is possible to create process hypotheses regarding snake origins. (Caldwell & Albino, 2001, p. 212).

> Interestingly, the two limbed marine snakes do not form a clade, but rather comprise the two most basal snake lineages; this is consistent with a marine origin for snakes (e.g., Nopcsa, 1923; Lee & Caldwell, 2000). The large size and absence of burrowing adaptations in the next two snake lineages (madtsoiids and *Dinilysia*) also argue against a subterranean origin (Scanlon & Lee, 2000). (Lee & Scanlon, 2002a, p. 386).

> Phylogenetic analysis suggests that *Mesoleptos* is the nearest relative of snakes; this phylogenetic position is consistent with its morphology being

intermediate between typical marine squamates (e.g. mosasauroids) and primitive marine snakes (pachyophiids). (Lee & Scanlon, 2002b, p. 131).

the snakes may have originated in a marine environment. This hypothesis is supported by the fact that the Mosasauroidea, Cretaceous lizards also markedly adapted to marine life, are widely recognized as the group most closely related to the snakes (e.g. Lee, 1997; Caldwell, 1999; Lee & Caldwell, 2000; see, however, the divergent views of Rieppel & Zaher, 2000). (Rage & Escullié, 2003, p. 8).

Combined morphological and molecular analyses also still unite marine lizards with snakes. Thus, the molecular data do not refute the phylogenetic evidence for a marine origin of snakes. (Lee, 2005a, p. 227).

That rationale is present in the claims of other ecologies and fossils:

The primitive, fossorial snake genera *Leptotyphlops* and *Typhlops* are basal to the other snakes and provide tentative molecular evidence in support of a fossorial or subfossorial origin of limblessness for the suborder Serpentes. (Forstner, 1995, p. 93).

Nonetheless, the significant exclusion of snakes from varanoids is in itself sufficient to support a terrestrial origin of snakes, because no other marine connection has been suggested or is likely. (Vidal & Hedges, 2004, p. 226).

The stem snake *Najash* is terrestrial, arguing against a marine origin, yet the animal's large size (~2m in length) seems inconsistent with the hypothesis that snakes evolved from burrowing forms. (Longrich et al., 2012, p. 205).

in combination with the recently revised phylogenetic position of the limbed Tethyan marine snakes (Simoliophiidae; e.g., *Haasiophis terrasanctus* [9], *Eupodophis descouensis* [10], and *Pachyrachis problematicus* [11]) as nested within Alethinophidia (rather than representing stem snakes) [4,8], offers compelling evidence against the marine origin hypothesis for snakes. (Hsiang et al., 2015, p. 2).

Nevertheless, our analyses do not support a marine origin for snakes, since we show that the earliest snake lineages are the burrowing scolecophidians" (Reeder et al., 2015, p. 11).

combined with its [*Najash*] hypothesized basal position as the sister group to all living snakes, has made it a crucial taxon for every recent study dealing with the origin and early evolution of snakes. (Garberoglio et al., 2019b, p. 1).

Many studies in the field of the early ecology and morphology of snakes became almost exclusively focused on finding the sister-group or sister-taxon of all other snakes, thereafter reducing, oversimplifying, and confusing its evolutionary history.

The second conceptual issue is assuming that one can equate the combination of phenotypic traits in fossils with those expected in their ancestors. Traditionally, changes in the fossil record have been seen as mostly gradual and so perfect transitional forms would be expected, or assumed, for most lineages through time - a classical example is the fossil record of horses (Simpson, 1944, 1953).

Such views on morphological evolution were shown to be simplistic (Orlando, 2013; MacFadden, 2005). That kind of disregard of evolutionary complexity contributed to the expectation of perfect fossil bridges (half-something/half-something else) or the so-called "missing links". The misleading notion of “missing links” dates back at least to the 18th century, being then co-opted to evolution (Olson, 1981). That happened because some combined their linear thinking about evolution with a gradual mode of phenotypic and evolutionary change. Reminiscences of this limited notion about morphological evolution can be unfortunately identified in the recent peer-reviewed scientific literature. Coates and Ruta (2000) were provocative in their review about the origin of snakes with the following subtitles: "Fossils: perfect missing link…” (p. 503) and “but perhaps not as primitive as their legs suggest?" (p. 504).

This superficial view of the fossil record and its reductionist connection with purely gradual phenotypic evolution have been insightfully criticized based on the variable relationships existing between the tempo and mode of evolution in the broader sense of the paleontological record (Eldredge & Gould, 1972; Gould & Eldredge, 1977; Gould, 2002). For instance, the punctuated equilibrium model of evolution opened a venue for discussions on assumptions about morphological evolution (Gould & Eldredge, 1993). It initially combined allopatric speciation (Mayr, 1963), stasis, and species selection to explain phenotypic changes between fossil species. In small populations, rapid evolutionary changes can take place whereas in large populations, stabilizing selection would keep species evolving around a mean phenotypic shape. That evolutionary model demonstrated that evolution could proceed by variable rates. Benton and Pearson (2001) found that vertebrates commonly have punctuated patterns of diversification, with periods of rapid speciation followed by long-term stasis of species lineages. That notion has been though partially challenged (Harmon *et al.,* 2010). As a take-on message, phyletic gradualism should not be used as a granted condition that would allow a researcher to deduce ancestral morphologies from fossils alone that were dated closely to the estimated age of the most recent common ancestor of a group. Even within a short period, the difference between descendant lineages and ancestral

forms can be dramatic (Gould & Eldredge, 1977, 1993). Thus, equating fossils to ancestors "reflects the persistent bias for gradualism still deeply embedded in paleontological thought" (Gould & Eldredge, 1977). Nowadays, rates of evolution are commonly investigated in phylogenies regardless of evoking or not the punctuated equilibrium (Watanabe et al., 2019, Simões et al., 2020).

Misconceptions such as equating fast morphological changes with saltationism (Goldschmidt, 1960) and quantum evolution (Simpson, 1944, 1953), in addition to public discussions against creationists, can often lead to simplistic interpretations by evolutionists of the tempo and mode of phenotypic evolution so to fit a strict phyletic process (Sterelny, 2003; Shubin, 2009; Coyne, 2010; Dawkins, 2009). Furthermore, disregarding developmental bias (constraints, modularity, and correlation of traits) diminishes our current understanding that complex traits often show non-random variation (Alberch, 1980; Gould, 2002; Salazar-Ciudad & Jervall, 2005). Variation in simple phenotypes fit to neo-Darwinian assumptions but complex phenotypes occupy the morphospace like islands (Salazar-Ciudad & Jervall, 2005).

In morphodynamic mechanisms, induction and morphogenetic processes act in synergy. In morphostatic mechanisms, induction events happen before morphogenetic mechanisms, and thus growth cannot influence the induction of a pattern. In the former, small changes in a gene product can result in highly non-linear effects that can produce new morphological structures with higher disparity (Salazar-Ciudad et al., 2003). More continuous phenotypic variation can be produced by morphostatic mechanisms, but those are expected in simpler univariate traits (Salazar-Ciudad et al., 2003). Skull shape is intrinsically multivariate. Sustained adaptation is seemingly only possible from simpler phenotypes according to models of phenotype–fitness maps (Salazar-Ciudad & Marín-Riera, 2013).

Classical textbooks and science magazines have commonly spread the notion of orthodoxical continuous rate of gradual evolution, including tales of "missing links" linked to *Archaeopteryx*, *Australopithecus, and Tiktaalik*. They are representatives of more complex phenotypic landscapes of poorly represented lineages (Niedźwiedzki et al., 2010; Harari 2011; Godefroit et al., 2013; Balanoff et al., 2013). Their once thought unique transitional characters are now known to be shared with several other species from distantly related lineages. Taxa with a mix of plesiomorphic and apomorphic traits do exist, but fossils cannot be directly equated to the total ancestral morphology or shape.

This reductionist gradualism was early noticed by Thompson (1917):

> Mr. Heilmann tells me that he has tried, but without success, to obtain a transitional series between the human skull and some prehuman, anthropoid type, which series (as in the case of the Equidae) should be

> found to contain other known types in direct linear sequence. It appears impossible, however, to obtain such a series, or to pass by successive and continuous gradations through such forms as *Mesopithecus*, *Pithecanthropus*, *Homo neanderthalensis*, and the lower or higher races of modern man. The failure is not the fault of our method. It merely indicates that no one straight line of descent, or of consecutive transformation, exists; but on the contrary, that among human and anthropoid types, recent and extinct, we have to do with a complex problem of divergent, rather than of continuous, variation. And in like manner, easy as it is to correlate the baboon's and chimpanzee's skulls severally with that of man, and easy as it is to see that the chimpanzee's skull is much nearer to the human type than is the baboon's, it is also not difficult to perceive that the series is not, strictly speaking, continuous, and that neither of our two apes lies *precisely* on the same direct line or sequence of deformation by which we may hypothetically connect the other with man. (pp. 773-774).

This debate seems to have had limited impact on conceptual thinking, and assumptions on phenotypic evolution, but also in the lexicon of several studies about the early ecological origin of snakes, as are exemplified below:

> no obvious **intermediate stages** have been identified. Here we present evidence that mosasaurs: large, extinct marine lizards related to snakes, represent **a crucial intermediate stage**. Thus, **intermediate** morphologies in snake skull evolution should perhaps be sought not in small burrowing lizards, as commonly assumed, but in large marine forms. (Lee et al., 1999a, p. 656).

> For a long time, the nearest lizard relatives of snakes remained uncertain, and **no transitional taxa were known**. Recently, however, several independent studies have concluded that large marine mosasauroids are the nearest relatives of snakes (Scanlon, 1996; Lee,1991,1998; Caldwell, 1998; Rage, 1997), and **a well-preserved transitional taxon**, *Pachyrhachis problematicus,* has been identified (Caldwell and Lee, 1997). Here, we discuss the ecology of this **important intermediate** and its implications for the evolutionary biology of snake origins. (Scanlon, 1999, p. 127).

> Phylogenetic analysis suggests that *Mesoleptos* is the nearest relative of snakes; **this phylogenetic position is consistent** with its morphology being **intermediate between** typical marine squamates (e.g. mosasauroids) and primitive marine snakes (pachyophiids). (Lee and Scanlon 2002, p. 131).

> [on the outgroup position of *Najash*] This is an intriguing phylogenetic

> proposal considering that *Najash* is **only slightly older** than *Dinilysi*a (Upper Turonian versus Santonian). (Caldwell &Calvo, 2008, p. 360).
>
> *Coniophis* therefore represents **a transitional snake**, **combining a snake-like body and a lizard-like head**. (Longrich et al., 2012, p. 205).
>
> These snakes [*Pachyrhachis problematicus* and *Eupodophis descouensi*] would represent **almost ideal intermediates** between the body plan of dolichosaurs and that of more advanced snakes. (Palci et al., 2013b, p. 1329).
>
> Thus, **the importance of fossil intermediates** for illuminating macroevolutionary processes **cannot be understated**.(...) **Transitional fossils are therefore invaluable.** (Hsiang et al., 2015, p. 15).

This rationale of assumed "perfect" intermediate fossils, and their immediate connection to ancestral ecologies, is partially understandable because fossils are factual and show morphological change *in situ* while ancestral estimations are probabilistic. However, it is important to stress that fossils should neither be equated to ancestors nor being used to directly imply the ancestral morphology and ecology, particularly considering the poor fossil record of snakes. Illustrating, the ancestral-state estimations done in this dissertation found that the ecology of the ancestor of Pythonomorpha (uniting large marine lizards with snakes) was terrestrial. This is compatible with the earliest ecological hypothesis for the ancestor of snakes (Cope, 1869). Cope did not imply that the relationship of snakes with marine mosasauroids was indicative of a marine origin of snakes. On the other hand, contemporary authors with robust phylogeentic methods did so (e.g., Caldwell & Lee, 1997). It is not necessarily methodological; the issue is firstly conceptual. It is about how you see the world, as introduced in this dissertation by Jacob (1977)'s iconic extract from his publication "Evolution and Tinkering".

Thereafter, the problem of the ecological origin of snakes is also one of deduction and induction. Extrapolating that the snake ancestor has a certain ecology based on the ecology of the sister-group or a few fossils is the same as saying "this swan is the oldest swan cousin found to date and it is white, so the ancestor of all other swans must be white". That assertion is not necessarily reasonable because it assumes that ecological and morphological changes did not take place in the sister lineage since the separation from the common ancestor with the related lineage. Moreover, that extrapolation reflects a static view of evolution. This is so problematic that one might imply that because scolecophidian snakes are commonly retrieved as the sister-group of alethinophidians, or shown on the left side of the topology, the ancestor of snakes had to look like an extant blind snake. That is most likely not the case for blind snakes, as supported by the

results shown here, previous anatomical studies, and recently also by the fossil record (see section 5.2).

On the other hand, induction is rational thinking commonly associated with scientific practices such as the probabilistic access of the ancestral states. Ancestral state estimation (being parsimony, maximum likelihood, or Bayesian) provides probabilistic values that are based on the entire data (Ronquist, 2004). Evidently, as with any probabilistic method, it has important limitations and might not be reliable in certain cases (e.g., Losos, 1999; Oakley & Cunningham, 2000; Polly, 2001; Webster & Purvis, 2002). Yet, based on multiple cases in which it is convincing or the best-possible evidence, it has long become a powerful analytical resource in comparative biology (Cunningham et al., 1998). That is particularly the case if researchers have fossil remains available to contrast with estimations (Polly, 2001). Future fossil snake findings can be further contrasted with estimated skull shape ancestors and sizes. The expectation is that small and fossorial-like forms will be mostly recovered in the Lower Cretaceous while higher proportions of terrestrial-like forms in the Jurassic. Marine forms are less likely to be found. Marine forms would have likely evolved between the Lower and Upper Cretaceous.

In this dissertation and accompanying papers, several sources of evidence were sought in support of an ecological scenario for the origin and radiation of snakes - which is known as eliminative induction. Just to illustrate, the probability of each node was calculated through an independent method - discriminant function analysis - while the ancestral shape was estimated by squared-change parsimony. Ancestral-state estimations produced likelihoods that supported a terrestrial to fossorial transition between deep and crown snake ancestors. These estimations matched patterns of morphospace occupation, fossils distribution and sizes, and skull development. Earlier studies that employed ancestral-state estimation of qualitative traits did not find strong support for any of the ecological hypotheses, that likely due to sampling issues, low phylogenetic signal, and the quantitative nature of the form-and-function relationship (Hsiang et al., 2015; Wenerburg et al., 2015). The skull shapes of lizards and snakes were found here to have a significant phylogenetic signal but also retained ecological signatures. So far, no other study has retrieved contrary hypotheses from the ones presented here based on new data or analyses. Instead, studies have supported our findings based on complementary quantitative approaches (e.g., Watanabe et al., 2019).

The last issue is the misstatement of hypotheses that can make them unfalsifiable. Pooper (1959) wrote on the issue of dysfunctional hypotheses that "(Thus the statement, "It will rain or not rain here tomorrow" will not be regarded as empirical, simply because it cannot be refuted; whereas the statement, "it will rain here tomorrow" will be regarded as empirical.)" (p. 19).

For instance, a marine and terrestrial ancestor (Caldwell et al., 2015). One

could though code the data for semi-aquatic or semi-terrestrial.

Redirecting attention to criticism raised by Caldwell (2019), he claimed that other studies in the field of snake evolution have systematically ignored his proposed terrestrial hypothesis. His last amended hypothesis is not testable because it cannot be falsified. On the other hand, Scanlon et al. (1999), Lee et al. (1999), Scanlon and Lee (2000) have clearly stated that the snake ancestor could have been marine or terrestrial, thus an undefined but testable state. Furthermore, this dissertation opposes previous simplistic ecological scenarios like fossorial-to-fossorial, terrestrial-to-terrestrial, and marine-to-marine. It also rejects marine-to-fossorial and marine-to-terrestrial hypotheses. This present body of work favors the evolution of terrestrial-to-fossorial ancestors.

Finally, Caldwell (2019) claimed that my research was teleological by stating that "The outcomes of which [teleology] still burden progress if progress is measured in proposing new paradigms and scenarios for snake lizard evolution (e.g., Da Silva et al., 2018)." (p. 197). The adaptationist program, generally known by the Panglossian paradigm, has long been re-thought in evolution (Gould & Lewontin, 1979). Finding correlation of form (shape) and function (ecology) is not the same as stating causation or that a given structure evolved for a certain function. My results showed that fossorial forms are more convergent than expected under a neutral evolution model, likely indicating selective demands associated with living underground. The fossorial skull shape is particularly distinguishable from all the other ecologies, and the crown snake ancestor was recovered to have a skull shape compatible with fossoriality. The overall significant association between form and function allowed us to estimate function based on form, but it does not tell us about how each association came into existence. On the other hand, I did provide insights based on the feedback-loop mechanism between natural selection and development as detailed in this dissertation. All findings and the proposed model can be tested. Kuhn (1970) is again insightful when he wrote that "As has repeatedly been emphasized before, no theory ever solves all the puzzles with which it is confronted at a given time; nor are the solutions already achieved often perfect". (p. 146).

Darwin (1959) had startlingly clarity:

> I by no means expect to convince experienced naturalists whose minds are stocked with a multitude of facts all viewed, during a long course of years, from a point of view directly opposite to mine. (...) but I look with confidence to the future. (p. 482).

This dissertation is understood to have provided new insights into an old scientific problem: the ecological origin and radiation of snakes. I tried my best to acknowledge every major contribution to the field before my efforts. I apologize to the authors of any study that might not have been

cited. I would like to express my deep respect and admiration for the work developed by other researchers in the field of snake evolution, including those I have criticized or replied to their criticism. This dissertation would have been far more limited without their extensive prior contributions. Finally, this dissertation welcomes future studies to evaluate the results and models presented here, seeking to the maximum to refute them.

6) LIMITATIONS AND FUTURE DIRECTIONS

As for scientific research, this research program has limitations. I presented in the text that limitations such as differences between 2D and 3D were circumvented, and results were convergent. Another limitation of landmark-based geometric morphometrics is that a considerable number of anatomical positions might not be reliably asserted, for example, along curvatures (Adams et al., 2004). In this case, other methods can be employed such as semi-landmarks (Watanabe et al., 2019) and landmark-free methods such as Particle-based models of shape such as ShapeWorks (Oguz et al., 2016) and Procrustes Surface Analysis (Pomidor et al., 2016).

This dissertation did not consider sexual shape dimorphism, which might be relevant even for macroevolutionary studies as it has been shown for *Anolis* lizards (e.g., Butler et al., 2007). For example, new studies might estimate that early snakes were sexually dimorphic and that morphospace might differ between sexes.

New fossil findings should be contrasted to our skull evolution model and ecological origin of snakes (e.g., *Najash*: Garberoglio et al., 2019a,b), but so far, the most relevant geological period (Lower Cretaceous) offers little opportunity for such comparisons as there are no snake skull fossils described yet. Future studies should also focus on assembling 3D reconstructions of known fossil snakes (e.g., Palci et al., 2018, Polcyn et al., 2005), although I expect that results will fit patterns recovered here.

Other ancestral-state estimation methods should be employed, for example, see Hsiang et al. (2015) and data contrasted with new fossil findings, including the presence/absence of vertebral remains. In this regard, a combination of the skull and body shape evolution should be sought in the future in more detail, which was beyond the scope of this dissertation.

The causal relationship of form and function should also be further investigated with other techniques such as Finite Element Analysis (FEA) (e.g., Simões et al., 2016) that would allow for modeling of skull load and its relationship with habitat and diet. Other functional studies could better define the role of shape changes of the quadrate and the evolution of large gape size.

Finally, the relationship between cranial and brain shape might

prove valuable in clarifying the early ecology of snakes (e.g., Fabbri et al., 2017), as shape analysis of brain parts seem to indicate that (Macrì et al., 2019).

7) CONCLUSIONS

In the tradition of S. J. Gould, a source of inspiration for me as a scientist, my scientific approach, and research agenda, I conclude this dissertation with general remarks:

- The correlation between skull shape and habitat allowed for paleoecological predictions of ancestors controlled by phylogenetic structure.
- Fossorial snakes and lizards represent a striking example of a deep-evolutionary pattern of convergence between distantly related and geographically separated lineages that will contribute to homoplasy in phylogenetic studies.
- The earliest snake ancestor was likely a terrestrial without fossorial behavior and the crown snake ancestor was most likely a fossorial, thus the marine origin of snakes can be excluded with high levels of confidence. Allometry was crucial in the early phase.
- Snake skulls develop faster than those of lizards, both shape and ossification rates are higher, including in the fossil record (e.g., *Dinilysia*), with strong implications for phenotypic innovation, species/ecological radiation in later phases.
- Evolutionary innovations in the shape of the skull, particularly the quadrate bone, shifted the relevance of habitat in early snake evolution to diet in the later appearance of boas, pythons, and caenophidian snakes. They created conditions for morphological, species, and ecological radiations.
- Misconceptions about phylogenies and fossils in relationship to ancestral-state estimation and the mode of phenotypic evolution are highly encouraged to be addressed. Future studies and the field of snake evolution will benefit from a strong conceptual framework aligned with modern scientific and biological thought.
- Mathematical formalization and large-scale data have arrived to stay. Future studies should make full use of such scientific advancements to test hypotheses and avoid narratives based on a few sources of data or single species.

8) REFERENCES

Abdala, F., Lobo, F., & Scrocchi, G. (1997). Patterns of ossification in the skeleton of *Liolaemus quilmes* (Iguania: Tropiduridae). *Amphibia Reptilia, 18*, 75–83. doi: 10.1163/156853897X00323

Abzhanov, A. (2013). Von Baer's law for the ages: Lost and found principles of developmental evolution. *Trends in Genetics*, *29*(12), 712–722. https://doi.org/10.1016/j.tig.2013.09.004

Abzhanov, A. (2017). The old and new faces of morphology: The legacy of D'Arcy Thompson's 'theory of transformations' and 'laws of growth.' *Development*, *144*(23), 4284–4297. https://doi.org/10.1242/dev.137505

Abzhanov, A., Rodda, S. J., McMahon, A. P., & Tabin, C. J. (2007). Regulation of skeletogenic differentiation in cranial dermal bone. *Development*, *134*(17), 3133–3144. https://doi.org/10.1242/dev.002709

Adams, D. C. (2014). A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology*, *63*(5), 685–697. https://doi.org/10.1093/sysbio/syu030

Adams, D. C., & Collyer, M. L. (2009). A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution*, *63*(5), 1143–1154. https://doi.org/10.1111/j.1558-5646.2009.00649.x

Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*(4), 393–399. https://doi.org/10.1111/2041-210X.12035

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution.' *Italian Journal of Zoology*, *71*(1), 5–16. https://doi.org/10.1080/11250000409356545

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2013). A field comes of age: Geometric morphometrics in the 21st century. *Hystrix, 24*(1), 7–14. https://doi.org/10.4404/hystrix-24.1-6283

Aguilar-Puntriano, C., Avila, L. J., De la Riva, I., Johnson, L., Morando, M., Troncoso-Palacios, J., Wood, P. L., & Sites, J. W. (2018). The shadow of the past: Convergence of young and old South American desert lizards as measured by head shape traits. *Ecology and Evolution*, *8*(23), 11399–11409. https://doi.org/10.1002/ece3.4548

Al Mohammadi, A. G. A., Khannoon, E. R., & Evans, S. E. (2020). The development of the osteocranium in the snake Psammophis sibilans (Serpentes: Lamprophiidae). *Journal of Anatomy*, *236*(1), 117–131. https://doi.org/10.1111/joa.13081

Alberch, P. (1980). Ontogenesis and morphological diversification. *American Zoologist*, *20*(4), 653-667.

Alberch, P. (1985). Problems with the interpretation of developmental sequences. *Systematic Zoology, 34*(1), 46. https://doi.org/10.2307/2413344

Alberch, P. (1989). The logic of monsters: Evidence for internal constraint in development and evolution. *Geobios, 22(*2), 21–57. https://doi.org/10.1016/S0016-6995(89)80006-3

Alberch, P. (1991). From genes to phenotype: dynamical systems and evolvability. *Genetica, 84*(1), 5–11. https://doi.org/10.1007/BF00123979

Alberch, P., Gould, S. J., Oster, G. F., & Wake, D. B. (1979). Size and shape in ontogeny and phylogeny. *Paleobiology, 5*(3), 296–317. https://doi.org/10.1017/s0094837300006588

Albert, E. M., San Mauro, D., García-París, M., Rüber, L., & Zardoya, R. (2009). Effect of

taxon sampling on recovering the phylogeny of squamate reptiles based on complete mitochondrial genome and nuclear gene sequence data. *Gene, 441*(1–2), 12–21. https://doi.org/10.1016/j.gene.2008.05.014

Albino, A. M. (2011). Evolution of Squamata Reptiles in Patagonia based on the fossil record. *Biological Journal of the Linnean Society, 103*(2), 441–457. https://doi.org/10.1111/j.1095-8312.2011.01691.x

Albino, A. M., & Brizuela, S. (2014). An overview of the South American fossil squamates. *Anatomical Record, 297*(3), 349–368. https://doi.org/10.1002/ar.22858

Albino, A.M., Carrillo-Briceño, J. D., & Neenan, J. M. (2016). An enigmatic aquatic snake from the Cenomanian of Northern South America. *PeerJ, 2016*(5). https://doi.org/10.7717/peerj.2027

Altman, N., & Krzywinski, M. (2016). Points of significance: P values and the search for significance. *Nature Methods, 14*(1), 3–4. https://doi.org/10.1038/nmeth.4120

Alvarez, B., Lions, M., & Calamante, C. (2005). Biologia reproductiva y desarrollo del esqueleto de *Polychrus acutirostris* (Iguania, Polychrotidae). *Facena, 21*(1), 3–27. http://exa.unne.edu.ar/revisfacena/21/3-27.pdf

Amrhein, V., Greenland, S. G., & McShane, B. (2019). Retire statistical significance. *Nature, 567*, 305–307.

Andjelković, M., Tomović, L., & Ivanović, A. (2017). Morphological integration of the kinetic skull in *Natrix* snakes. *Journal of Zoology, 303*(3), 188–198. https://doi.org/10.1111/jzo.12477

Andrews, R. M., Brandley, M. C., & Greene, V. W. (2013). Developmental sequences of squamate reptiles are taxon specific. *Evolution and Development, 15*(5), 326–343. https://doi.org/10.1111/ede.12042

Apesteguía, S., & Novas, F. E. (2003). Large Cretaceous sphenodontian from Patagonia provides insight into lepidosaur evolution in Gondwana. *Nature, 425*(6958), 609–612. https://doi.org/10.1038/nature01995

Apesteguía, S., & Zaher, H. (2006). A Cretaceous terrestrial snake with robust hindlimbs and a sacrum. *Nature, 440*(7087), 1037–1040. https://doi.org/10.1038/nature04413

Arias, F., & Lobo, F. (2006). Patrones de osificación en *Tupinambis merianae* y *Tupinambis rufescens* (Squamata: Teiidae) y patrones generales en Squamata. *Cuadernos de Herpetologia, 20*(1), 3–23.

Arrigo, M. I., De Oliveira Vilaca, L. M., Fofonjka, A., Srikanthan, A. N., Debry, A., & Milinkovitch, M. C. (2019). Phylogenetic mapping of scale nanostructure diversity in snakes. *BMC Evolutionary Biology, 19*(1), 1–20. https://doi.org/10.1186/s12862-019-1411-6

Arthur, W. (2006). D ' Arcy Thompson and the theory of transformations. *Genetics, 7*(1958), 7–12.

Balanoff, A. M., Bever, G. S., Rowe, T. B., & Norell, M. A. (2013). Evolutionary origins of the avian brain. *Nature*, *501*(7465), 93–96. https://doi.org/10.1038/nature12424

Ball, P. (2016). *Patterns in Nature: Why the Natural World looks the Way it Does*. University of Chicago Press.

Banavar, J. R., Cooke, T. J., Rinaldo, A., & Maritan, A. (2014). Form, function, and evolution of living organisms. *Proceedings of the National Academy of Sciences of the United States of America, 111*(9), 3332–3337. https://doi.org/10.1073/pnas.1401336111

Băncilă, R., Gelder, I. V., Rotteveel, E., Loman, J., & Arntzen, J. W. (2010). Fluctuating asymmetry is a function of population isolation in island lizards. *Journal of Zoology,* 282(4), 266-275. https://doi.org/10.1111/j.1469-7998.2010.00736.

Bardua, C., Felice, R. N., Watanabe, A., Fabre, A.-C., & Goswami, A. (2019). A Practical Guide to Sliding and Surface Semilandmarks in Morphometric Analyses. *Integrative Organismal Biology, 1*(1). https://doi.org/10.1093/iob/obz016

Barresi, M. J. F., & Gilbert, S. F. (2020). *Developmental Biology* (12th Ed.). Sinauer Associates, Oxford University Press.

Barrie, D. J. (1990). Skull elements and additional remains of the pleistocene boid snake *Wonambi naracoortensis*. *Mem. Qd. Mus., 28*(1), 139-151.

Barros, F. C., Herrel, A., & Kohlsdorf, T. (2011). Head shape evolution in Gymnophthalmidae: Does habitat use constrain the evolution of cranial design in fossorial lizards? *Journal of Evolutionary Biology, 24*(11), 2423–2433. https://doi.org/10.1111/j.1420-9101.2011.02372.x

Barton, N. H., Briggs, D., Eisen, J. A., Goldstein, D. B., & Patel, N. H. (2007). *Evolution*. Cold Spring Harbor Laboratory Press.

Baszio, S. (2004). *Messelophis ermannorum* n. sp., eine neue Zwergboa (Serpentes: Boidae: Tropidopheinae) aus dem Mittel-Eozän von Messel. *Courier Forschungsinstitut Senckenberg, 252*: 67–77.

Baum, D. A., Smith, S. D. W., & Donovan, S. S. S. (2005). The tree-thinking challenge. *Science, 310*(5750), 979–980. https://doi.org/10.1126/science.1117727

Bellairs A. D. A., & Underwood, G. (1951). The origin of snakes. *Biological Reviews, 26*(2), 193–237. https://doi.org/10.1111/j.1469-185X.1951.tb00646.x

Bellairs, A. d'A., & Kamal, A. El-D. M. (1981). The chondrocranium and the development of the skull in recent reptiles. In C. Gans & T. S. Parsons (Eds.), *Biology of the Reptilia* (Volume 11, Morphology F, pp. 1-263). Academic Press.

Benton, M. J., & Pearson, P. N. (2001). Speciation in the fossil record. *Trends in Ecology and Evolution, 16*(7), 405–411. https://doi.org/10.1016/S0169-5347(01)02149-8

Benyus, J. M. (1997). *Biomimicry: Innovation Inspired by Nature*. HarperCollins.

Bhullar, B. A. S., Marugán-Lobón, J., Racimo, F., Bever, G. S., Rowe, T. B., Norell, M. A., & Abzhanov, A. (2012). Birds have paedomorphic dinosaur skulls. *Nature, 487*(7406), 223–226. https://doi.org/10.1038/nature11146

Bhushan, B. (2009). Biomimetics: Lessons from nature - an overview. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 367*(1893), 1445–1486. https://doi.org/10.1098/rsta.2009.0011

Bininda-Emonds, O. R. P., Jeffery, J. E., & Richardson, M. K. (2003). Inverting the hourglass: Quantitative evidence against the phylotypic stage in vertebrate development. *Proceedings of the Royal Society B: Biological Sciences, 270*(1513), 341–346. https://doi.org/10.1098/rspb.2002.2242

Blomberg, S. P., & Garland, T. (2002). Tempo and mode in evolution: Phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology, 15*(6), 899–910. https://doi.org/10.1046/j.1420-9101.2002.00472.x

Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution, 57*(4), 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Blomberg, S. P., Lefevre, J. G., Wells, J. A., & Waterhouse, M. (2012). Independent contrasts and PGLS regression estimators are equivalent. *Systematic Biology, 61*(3), 382–391. https://doi.org/10.1093/sysbio/syr118

Boback, S. M., Dichter, E. K., & Mistry, H. L. (2012). A developmental staging series for the African house snake, *Boaedon (Lamprophis) fuliginosus*. *Zoology, 115*(1), 38–46. https://doi.org/10.1016/j.zool.2011.09.001

Bolkay, S. J. (1925). *Mesophis nopcsai* n.g. n.sp. ein neues, schlangenähnliches reptil aus der unteren Kreide (Neocom) von Bilek-Selista (Ost-Hercegovina). *Glasnik zemaljskog Muzeja u Bosni i Hercegovini, 37,* 125-135.

Bon, M., Bardua, C., Goswami, A., & Fabre, A. C. (2020). Cranial integration in the fire salamander, *Salamandra salamandra* (Caudata: Salamandridae). *Biological Journal of the Linnean Society, 130*(1), 178–194. https://doi.org/10.1093/biolinnean/blaa020

Bookstein, F. L. (1989). Principal Warps: Thin-Plate Splines and the Decomposition of

Deformations. *IEEE Trans. Pattern Anal. Mach. Intell.*, *11*, 567-585.

Bookstein, F. L. (1991). *Morphometric Tools For Landmark Data: Geometry And Biology*. Cambridge University Press, New York.

Bookstein, F. L. (1998). A hundred years of morphometrics. *Acta Zool. Acad. Sci. Hung., 44*, 7-59.

Boughner, J. C., Buchtová, M., Fu, K., Diewert, V., Hallgrímsson, B., & Richman, J. M. (2007). Embryonic development of *Python sebae* - I: Staging criteria and macroscopic skeletal morphogenesis of the head and limbs. *Zoology, 110*(3), 212–230. https://doi.org/10.1016/j.zool.2007.01.005

Brakefield, P. M. (2006). Evo-devo and constraints on selection. *Trends in Ecology and Evolution, 21*(7), 362–368. https://doi.org/10.1016/j.tree.2006.05.001

Brandley, M. C., Huelsenbeck, J. P., & Wiens, J. J. (2008). Rates and patterns in the evolution of snake-like body form in squamate reptiles: Evidence for repeated re-evolution of lost digits and long-term persistence of intermediate body forms. *Evolution, 62*(8), 2042–2064. https://doi.org/10.1111/j.1558-5646.2008.00430.x

Brock, G. T. (1941). The skull of *Acontias meleagris*, with a study of the affinities between lizards and snakes. *Journal of the Linnean Society of London, Zoology, 41*(277), 71–88. https://doi.org/10.1111/j.1096-3642.1941.tb02289.x

Brock, G. T., Sc, M., & Oxon, D. P. (1929). Memoirs: On the development of the skull of *Leptodeira hotamboia*. *Journal of Cell Science, s2-73*(290), 289–331.

Broeckhoven, C., & Du Plessis, A. (2018). X-ray microtomography in herpetological research: A review. *Amphibia Reptilia, 39*(4), 377–401. https://doi.org/10.1163/15685381-20181102

Bruner, E., & Costantini, D. (2009). Head morphology and degree of variation in *Lacerta bilineata*, *Podarcis muralis* and *Podarcis sicula*. *International Journal of Morphology*, *27*(3), 667–676. https://doi.org/10.4067/s0717-95022009000300008

Bruner, E., Costantini, D., Fanfani, A., & Dell'Omo, G. (2005). Morphological variation and sexual dimorphism of the cephalic scales in *Lacerta bilineata*. *Acta Zoologica*, *86*(4), 245–254. https://doi.org/10.1111/j.1463-6395.2005.00206.x

Bruton, M. J. (2013). Arboreality, excavation, and active foraging: novel observations of radiotracked woma pythons *Aspidites ramsayi*. *Memoirs of the Queensland Museum – Nature, 56*(2): 313–329.

Burbrink, F. T., Grazziotin, F. G., Pyron, R. A., Cundall, D., Donnellan, S., Irish, F., Keogh, J. S., Kraus, F., Murphy, R. W., Noonan, B., Raxworthy, C. J., Ruane, S., Lemmon, A. R., Lemmon, E. M., & Zaher, H. (2020). Interrogating genomic-scale data for squamata (lizards, snakes, and amphisbaenians) shows no support for key traditional morphological relationships. *Systematic Biology, 69*(3), 502–520. https://doi.org/10.1093/sysbio/syz062

Bütikofer, L., Sacchi, R., Pupin, F., Pellitteri-Rosa, D., Razzetti, E., F. Pella, & Fasola, M.. (2018). Sexual dimorphism and allometry of the lacertid *Mesalina balfouri* (Bianford, 1881), endemic to the Archipelago of Socotra (Yemen). *Acta Medica Mediterranea*, 34, 583.

Butler, M. A., Sawyer, S. A., & Losos, J. B. (2007). Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature, 447*(7141), 202–205. https://doi.org/10.1038/nature05774

Caldwell, M. W. (1999). Squamate phylogeny and the relationships of snakes and mosasauroids. *Zoological Journal of the Linnean Society, 125*, 115-147.

Caldwell, M. W. (2000). On the phylogenetic relationships of *Pachyrhachis* within snakes: A response to Zaher (1998). *Journal of Vertebrate Paleontology, 20*(1), 187–190. https://doi.org/10.1671/0272-4634(2000)020[0187:OTPROP]2.0.CO;2

Caldwell, M. W. (2007). Snake phylogeny, origins, and evolution: the role, impact, and importance of fossils (1869–2006). In J. S. Anderson and H.-D. Sues (Eds.), *Major*

Transitions in Vertebrate Evolution (pp. 253–302). Indiana University Press, Bloomington and Indianapolis.

Caldwell, M. W. (2019). *The origin of snakes: Morphology and the fossil record*. CRC Press.

Caldwell, M. W., & Lee, M. S. Y. (1997). A snake with legs from the marine Cretaceous of the Middle East. *Nature, 386,* 705–709.

Caldwell, M. W., & Albino, A. (2002). Exceptionally preserved skeletons of the Cretaceous snake *Dinilysia patagonica* Woodward, 1901. *Journal of Vertebrate Paleontology, 22*(4), 861–866. https://doi.org/10.1671/0272-4634(2002)022[0861:EPSOTC]2.0.CO;2

Caldwell, M. W., & Albino, A. M. (2001). Palaeoenvironment and palaeoecology of three cretaceous snakes: *Pachyophis, Pachyrhachis*, and *Dinilysia*. *Acta Palaeontologica Polonica, 46*(2), 203–218. https://doi.org/10.7939/R3RZ8C

Caldwell, M. W., & Calvo, J. (2008). Details of a new skull and articulated cervical column of *Dinilysia patagonica* Woodward, 1901. *Journal of Vertebrate Paleontology, 28*(2), 349–362. https://doi.org/10.1671/0272-4634(2008)28[349:DOANSA]2.0.CO;2

Caldwell, M. W., Nydam, R. L., Palci, A., & Apesteguía, S. (2015). The oldest known snakes from the Middle Jurassic-Lower Cretaceous provide insights on snake evolution. *Nature Communications, 6*. https://doi.org/10.1038/ncomms6996

Camacho, J., Heyde, A., Bhullar, B. A. S., Haelewaters, D., Simmons, N. B., & Abzhanov, A. (2019). Peramorphosis, an evolutionary developmental mechanism in neotropical bat skull diversity. *Developmental Dynamics, 248*(11), 1129–1143. https://doi.org/10.1002/dvdy.90

Camp, C. L. (1923). Classification of lizards. *Bull. Am. Mus. Nat. Hist., 48*, 289-481.

Caprette, C. L., Lee, M. S. Y., Shine, R., Mokany, A., & Downhower, J. F. (2004). The origin of snakes (Serpentes) as seen through eye anatomy. *Biological Journal of the Linnean Society, 81*(4), 469–482. https://doi.org/10.1111/j.1095-8312.2003.00305.x

Carroll, S. B. (2005). *Endless Forms Most Beautiful: the New Science of Evo Devo and the Making of the Animal Kingdom*. W. W. Norton.

Casey, M. (2015). Oldest snake fossils show they thrived in the age of dinosaurs. CBS News.

Castoe, T. A., De Koning, A. P. J., Hall, K. T., Card, D. C., Schield, D. R., Fujita, M. K., Ruggiero, R. P., Degner, J. F., Daza, J. M., Gu, W., Reyes-Velasco, J., Shaney, K. J., Castoe, J. M., Fox, S. E., Poole, A. W., Polanco, D., Dobry, J., Vandewege, M. W., Li, Q., Schott, R. K., Kapusta, A., Minx, P., Feschotte, C., Uetz, P., Ray, D.A., Hoffmanni, F.G., Bogden, R., Smith, E.N., Chang, B.S. W., Vonk, F.J., Casewell, N.R., Henkel, C.V., Richardson, M. K., Mackessy, S.P., Bronikowski, A.M., Yandell, M., Warren, W.C., Secor, S.M., & Pollock, D. D. (2014). Erratum: The Burmese python genome reveals the molecular basis for extreme adaptation in snakes (Proceedings of the National Academy of Sciences of the United States of America (2013) 110, 51, (20645-20650) DOI: 10.1073/pnas. 1314475110). *Proceedings of the National Academy of Sciences of the United States of America*, 111(8), 3194. https://doi.org/10.1073/pnas.1324133111

Castoe, T. A., De Koning, A. P. J., Kim, H. M., Gu, W., Noonan, B. P., Naylor, G., Jiang, Z. J., Parkinson, C. L., & Pollock, D. D. (2009). Evidence for an ancient adaptive episode of convergent molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America, 106*(22), 8986–8991. https://doi.org/10.1073/pnas.0900233106

Čerňanský, A., & Stanley, E. L. (2019). The atlas–axis complex in Dibamidae (Reptilia: Squamata) and their potential relatives: The effect of a fossorial lifestyle on the morphology of this skeletal bridge. *Journal of Morphology, 280*(12), 1777–1797. https://doi.org/10.1002/jmor.21064

Chaplin, K., Sumner, J., Hipsley, C. A., & Melville, J. (2020). An integrative approach using phylogenomics and high-resolution X-ray computed tomography for species delimitation in cryptic taxa. *Systematic Biology*, *69*(2), 294–307.

https://doi.org/10.1093/sysbio/syz048

Cheverud, J. M. (1982). Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology, 59*(2), 139–149. https://doi.org/10.1002/ajpa.1330590204

Christensen, C. B., Christensen-Dalsgaard, J., Brandt, C., & Madsen, P. T. (2012). Hearing with an atympanic ear: Good vibration and poor sound-pressure detection in the royal python, *Python regius*. *Journal of Experimental Biology, 215*(2), 331–342. https://doi.org/10.1242/jeb.062539

Claude, J. (2008). *Morphometrics with R*. Springer-Verlag New York.

Coates, M., & Ruta, M. (2000). Nice snake, shame about the legs. *Trends in Ecology and Evolution, 15*(12), 506–507. https://doi.org/10.1016/s0169-5347(00)01999-6

Cohn, M. J., & Tickle, C. (1999). Developmental basis of limblessness and axial patterning in snakes. *Nature, 399*(6735), 474–479. https://doi.org/10.1038/20944

Collyer, M. L., & Adams, D. C. (2007). Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology, 88*(3), 683–692. https://doi.org/10.1890/06-0727

Collyer, M. L., & Adams, D. C. (2013). Phenotypic trajectory analysis: Comparison of shape change patterns in evolution and ecology. *Hystrix, 24*(1). https://doi.org/10.4404/hystrix-24.1-6298

Conrad, J. L. (2008). Phylogeny and systematics of squamata (reptilia) based on morphology. *Bulletin of the American Museum of Natural History, 310*, 1–182. https://doi.org/10.1206/310.1

Conway Morris, S. (2003). *Life's solution: inevitable humans in a lonely universe*. Cambridge University Press.

Cooke, S. B., & Terhune, C. E. (2015). Form, function, and geometric morphometrics. *Anatomical Record, 298*(1), 5–28. https://doi.org/10.1002/ar.23065

Cope, E. D. (1869). On the reptilian orders Pythonomorpha and Streptosauria. *Proc. Boston Soc. Nat. Hist., 12*, 250-266.

Cope, E. D. (1878). Professor Owen on the Pythonomorpha. *Bull. U.S. geol. SurŠ., 4*, 299-311.

Cope, E.D. (1864). On the characters of the higher groups of Reptilia Squamata—and especially of the Diploglossa. *Proceedings of the Academy of Natural Sciences of Philadelphia, 16*, 224—231.

Costantini, D., & Bruner, E. (2007). Head morphological variation in *Podarcis muralis* and Podarcis sicula: a landmark-based approach. Amphibia-Reptilia, 28(4), 566–573. https://doi.org/10.1163/156853807782152525

Costantini, D., Alonso, M. L., Moazen, M., & Bruner, E. (2010). The relationship between cephalic scales and bones in lizards: A preliminary microtomographic survey on three lacertid species. *Anatomical Record*, *293*(2), 183–194. https://doi.org/10.1002/ar.21048

Coyne, J. A. (2010). *Why Evolution Is True*. Penguin Books.

Crisp, M. D., & Cook, L. G. (2005). Do early branching lineages signify ancestral traits? *Trends in Ecology and Evolution, 20*(3), 122–128. https://doi.org/10.1016/j.tree.2004.11.010

Cundall, D. (1995). Feeding behaviour in *Cylindrophis* and its bearing on the evolution of alethinophidian snakes. *Journal of Zoology, 237*(3), 353–376. https://doi.org/10.1111/j.1469-7998.1995.tb02767.x

Cundall, D., & Greene, H. W. (2000). Feeding in snakes. In K. Schwenk (Ed.), *Feeding: form, function and evolution in tetrapod vertebrates* (pp. 293–333). San Diego: Academic Press.

Cundall, D., & Irish, F. (2008). The Snake Skull. In C. Gans, A. S. Gaunt, and K. Adler

(Eds.), *Biology of the Reptilia: The Skull of Lepidosauria* (Volume 20, Morphology H, pp 349-692). Society for the Study of Amphibians and Reptiles, Ithaca.

Cunningham, C. W., Omland, K. E., & Oakley, T. H. (1998). Reconstructing ancestral character states: A critical reappraisal. *Trends in Ecology and Evolution*, *13*(9), 361–366. https://doi.org/10.1016/S0169-5347(98)01382-2

Cuny, G., Jaeger, J. J., Mahboub, M., & Rage, J. -C. (1990). Les plus anciens serpents (Reptilia, Squamata) connus. Mise au point sur l'âge géologique des serpents de la partie moyenne du Crétacé. *C. R. Acad. Sci. Paris II, 311*, 1267–1272.

Cuthill, J. F. H., Guttenberg, N., & Budd, G. E. (2020). Impacts of speciation and extinction measured by an evolutionary decay clock. *Nature, 588*, 636-641. https://doi.org/10.1038/s41586-020-3003-4

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection, or the preservation of favoured races in the struggle for life (1st ed.)*. John Murray.

Davis, M. A., Douglas, M. R., Collyer, M. L., & Douglas, M. E. (2016). Deconstructing a species-complex: Geometric morphometric and molecular analyses define species in the Western Rattlesnake (Crotalus viridis). *PLoS ONE*, *11*(1). https://doi.org/10.1371/journal.pone.0146166

Daza, J. D., Herrera, A., Thomas, R., & Claudio, H. J. (2009). Are you what you eat? A geometric morphometric analysis of gekkotan skull shape. *Biological Journal of the Linnean Society, 97*(3), 677-707. https://academic.oup.com/biolinnean/article-abstract/97/3/677/2448155

De Beer, G. R. (1930). *Embryology and Evolution*. Clarendon Press, Oxford.

De Beer, G. R. (1951). *Embryos and Ancestors*. Clarendon Press, Oxford.

Di Pietro, D. O., Alcalde, L., & Williams, J. D. (2014). Nasal cartilages, hyobranchial apparatus, larynx, and glottal tubes in four species of Hydropsini (Serpentes: Dipsadidae: Xenodontinae). *Vertebrate Zoology, 64*(1), 103–111.

Di-Poï, N., Montoya-Burgos, J. I., & Duboule, D. (2009). Atypical relaxation of structural constraints in Hox gene clusters of the green anole lizard. *Genome Research, 19*(4), 602–610. https://doi.org/10.1101/gr.087932.108

Di-Poï, N., Montoya-Burgos, J. I., Miller, H., Pourquié, O., Milinkovitch, M. C., & Duboule, D. (2010). Changes in *Hox* genes structure and function during the evolution of the squamate body plan. *Nature, 464*(7285), 99–103. https://doi.org/10.1038/nature08789

Diaz, R. E., & Trainor, P. A. (2019) An integrative view of lepidosaur cranial anatomy, development, and diversification. In Ziermann J., Diaz Jr R., Diogo R. (Eds), *Heads, Jaws, and Muscles*. *Fascinating Life Sciences*. Springer, Cham. https://doi.org/10.1007/978-3-319-93560-7_9

Dickson, B. V., Clack, J. A., Smithson, T. R., & Pierce, S. E. (2020). Functional adaptive landscapes predict terrestrial capacity at the origin of limbs. *Nature*. https://doi.org/10.1038/s41586-020-2974-5

Dobzhansky, T. (1977). Nothing in biology makes sense except in the light of evolution. *The American Biology Teacher, 35*, 125-129.

Dollion, A. Y., Measey, G. J., Cornette, R., Carne, L., Tolley, K. A., da Silva, J. M., Boistel, R., Fabre, A. C., & Herrel, A. (2017). Does diet drive the evolution of head shape and bite force in chameleons of the genus Bradypodion? *Functional Ecology, 31*(3), 671–684. https://doi.org/10.1111/1365-2435.12750

Donihue, C. M., Herrel, A., Fabre, A. C., Kamath, A., Geneva, A. J., Schoener, T. W., Kolbe, J. J., & Losos, J. B. (2018). Hurricane-induced selection on the morphology of an island lizard. *Nature, 560*(7716), 88–91. https://doi.org/10.1038/s41586-018-0352-3

dos Santos, M. M., da Silva, F. M., Hingst-Zaher, E., Machado, F. A., Zaher, H. E. D., & Prudente, A. L. C. (2017). Cranial adaptations for feeding on snails in species of *Sibynomorphus* (Dipsadidae: Dipsadinae). *Zoology, 120*, 24–30 .

Drake, A. G., & Klingenberg, C. P. (2008). The pace of morphological change: Historical transformation of skull shape in St Bernard dogs. *Proceedings of the Royal Society B: Biological Sciences, 275*(1630), 71–76. https://doi.org/10.1098/rspb.2007.1169

Duboule, D. (1994). Temporal colinearity and the phylotypic progression: A basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development, 120*, 135–142.

Duit, R., Treagust, D. F., & Widodo, A. (2013). Teaching science for conceptual change – Theory and practice. In S. Vosniadou (Ed.), *International Handbook of Research in Conceptual Change* (pp. 487-503). Routledge, New York.

Eldredge, N., & Gould, S. J. (1972). Punctuated equilibria: an alternative to phyletic gradualism. In T. J. M. Schopf (Ed.), *Models in Paleobiology* (pp. 82-115). Freeman, Cooper and Co.

Erwin, D. H. (2015). Novelty and innovation in the history of life. *Current Biology, 25*(19), R930–R940. https://doi.org/10.1016/j.cub.2015.08.019

Erwin, D. H. (2017). The topology of evolutionary novelty and innovation in macroevolution. *Phil. Trans. R. Soc. B,* 372**,** 20160422. http://doi.org/10.1098/rstb.2016.0422

Espinoza, R. E., Wiens, J. J., & Tracy, C. R. (2004). Recurrent evolution of herbivory in small, cold-climate lizards: Breaking the ecophysiological rules of reptilian herbivory. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(48), 16819–16824. https://doi.org/10.1073/pnas.0401226101

Esquerré, D., & Keogh, J. S. (2016). Parallel selective pressures drive convergent diversification of phenotypes in pythons and boas. *Ecology Letters, 19*(7), 800–809. https://doi.org/10.1111/ele.12620

Esquerré, D., Sherratt, E., & Keogh, J. S. (2017). Evolution of extreme ontogenetic allometric diversity and heterochrony in pythons, a clade of giant and dwarf snakes. *Evolution, 71*(12), 2829–2844. https://doi.org/10.1111/evo.13382

Estes, R., De Queiroz, K., & Gauthier, J. (1988). Phylogenetic relationships within Squamata. In R. Estes and G. Pregill (Eds.), *Phylogenetic Relationships of the Lizard Families*. (pp. 119-281). Stanford University Press.

Estes, R., Frazzetta, T. H., & Williams, E. E. (1970). Studies of the fossil snake *Dinilysia patagonica* Woodward: Part I. Cranial morphology. *Bull. Mus. Comp. Zool., 140*(2), 25-74.

Evans, S. E. (1994). A new anguimorph lizard from the Jurassic and Lower Cretaceous of England. *Palaeontology, 37*(1), 33–49.

Evans, S. E. (2003). At the feet of the dinosaurs: The early history and radiation of lizards. *Biological Reviews of the Cambridge Philosophical Society, 78*(4), 513–551. https://doi.org/10.1017/S1464793103006134

Evans, S. E. (2008). The skull of lizards and tuatara. The Snake Skull. In C. Gans, A. S. Gaunt, & K. Adler (Eds.), *Biology of the Reptilia: The Skull of Lepidosauria* (Volume 20, Morphology H, pp 1-348). Society for the Study of Amphibians and Reptiles, Ithaca.

Evans, S. E. (2015). Four legs too many? Science, *349*(6246), 374-375. DOI: 10.1126/science.aac5672

Eymann, J., Salomies, L., Macrì, S., & Di-Poï, N. (2019). Variations in the proliferative activity of the peripheral retina correlate with postnatal ocular growth in squamate reptiles. *Journal of Comparative Neurology, 527*(14), 2356–2370. https://doi.org/10.1002/cne.24677

Fabbri, M., Mongiardino Koch, N., Pritchard, A. C., Hanson, M., Hoffman, E., Bever, G. S., Balanoff, A. M., Morris, Z. S., Field, D. J., Camacho, J., Rowe, T. B., Norell, M. A., Smith, R. M., Abzhanov, A., & Bhullar, B. A. S. (2017). The skull roof tracks the brain during the evolution and development of reptiles including birds. *Nature Ecology and*

Evolution, 1(10), 1543–1550. https://doi.org/10.1038/s41559-017-0288-2

Fabre, A. C., Bickford, D., Segall, M., & Herrel, A. (2016). The impact of diet, habitat use, and behaviour on head shape evolution in homalopsid snakes. *Biological Journal of the Linnean Society, 118*(3), 634–647. https://doi.org/10.1111/bij.12753

Fabre, A. C., Cornette, R., Huyghe, K., Andrade, D. V., & Herrel, A. (2014). Linear versus geometric morphometric approaches for the analysis of head shape dimorphism in lizards. *Journal of Morphology, 275*(9), 1016–1026. https://doi.org/10.1002/jmor.20278

Fachini, T. S., Onary, S., Palci A., Lee M. S. Y.Lee, Bronzati, M., & Hsiou, A. S. (2020). Cretaceous Blind Snake from Brazil Fills Major Gap in Snake Evolution. *Iscience, 23*(12), 101834. https://doi.org/10.1016/j.isci.2020.101834

Feijó, A., Ge, D., Wen, Z., Xia, L., & Yang, Q. (2020). Divergent adaptations in resource-use traits explain how pikas thrive on the roof of the world. *Functional Ecology, 34*(9), 1826–1838. https://doi.org/10.1111/1365-2435.13609

Felsenstein, J. (1985). Phylogenies and the Comparative Method. *The American Naturalist, 125*(1), 1-15.

Fernandez, V., Buffetaut, E., Suteethorn, V., Rage, J. C., Tafforeau, P., & Kundrát, M. (2015). Evidence of egg diversity in squamate evolution from Cretaceous anguimorph embryos. *PLoS ONE, 10*(7), 7–9. https://doi.org/10.1371/journal.pone.0128610

Fisher, R. A. (1936). The use of multiple measurements in taxonomic problems. *Annals of Eugenics*, 7(2), 179–188. doi:10.1111/j.1469-1809.1936.tb02137.x

Forsman, A. (1996). An experimental test for food effects on head size allometry in juvenile snakes. *Evolution, 50*(6), 2536–2542. https://doi.org/10.1111/j.1558-5646.1996.tb03642.x

Forstner, M. R., S. K. Davis, & Arevalo , E.. (1995). Support for the hypothesis of anguimorph ancestry for the suborder serpentes from phylogenetic analysis of mitochondrial dna sequences. *Mol. Phylogenet. Evol., 4*(1), 93-102.

Frazzetta, T. H. (1970). Studies on the fossil snake *Dinilysia patagonica* Woodward. Part II. Jaw machinery in the earliest snakes. *Forma et Functio, 3*, 205–221.

Fry, B. G., Vidal, N., Norman, J. A., Vonk, F. J., Scheib, H., Ramjan, S. F. R., Kuruppu, S., Fung, K., Hedges, S. B., Richardson, M. K., Hodgson, W. C., Ignjatovic, V., Summerhayes, R., & Kochva, E. (2006). Early evolution of the venom system in lizards and snakes. *Nature, 439*(7076), 584–588. https://doi.org/10.1038/nature04328

Gabelaia, M., Tarkhnishvili, D., & Adriaens, D. (2018). Use of three-dimensional geometric morphometrics for the identification of closely related species of Caucasian rock lizards (Lacertidae: *Darevskia*). *Biological Journal of the Linnean Society*, 709–717. https://doi.org/10.1093/biolinnean/bly143

Galis, F., & Metz, J. A. J. (2001). Testing the vulnerability of the phylotypic stage: On modularity and evolutionary conservation. *Journal of Experimental Zoology, 291*(2), 195–204. https://doi.org/10.1002/jez.1069

Gans, C., & Monteiro, R. (2008). An Atlas of Amphisbaenian skull anatomy. In C. Gans, A. S. Gaunt and K. Adler (Eds.), *Biology of the Reptilia* (Volume 21, Morphology I, pp. 621-738). Society of the study of Amphibians and Reptiles.

Garberoglio, F. F., Apesteguía, S., Simões, T. R., Palci, A., Gómez, R. O., Nydam, R. L., E Larsson, H. C., Y Lee, M. S., & Caldwell, M. W. (2019a). New skulls and skeletons of the Cretaceous legged snake *Najash*, and the evolution of the modern snake body plan. Science Advances, *5*(11), eaax5833. DOI: 10.1126/sciadv.aax5833

Garberoglio, F. F., Gómez, R. O., Apesteguía, S., Caldwell, M. W., Sánchez, M. L., & Veiga, G. (2019b). A new specimen with skull and vertebrae of *Najash rionegrina* (Lepidosauria: Ophidia) from the early Late Cretaceous of Patagonia. *Journal of Systematic Palaeontology, 17*(18), 1313–1330. https://doi.org/10.1080/14772019.2018.1534288

Garland, T. Jr., Dickerman, A. W., Janis, C. M., & Jones, J. A. (1993). Phylogenetic analysis of covariance by computer simulation. *Systematic Biology,* 42(3), 265-292.

Gauthier, J. A., Kearney, M., Maisano, J. A., Rieppel, O., & Behlke, A. D. B. (2012). Assembling the squamate tree of life: Perspectives from the phenotype and the fossil record. *Bulletin of the Peabody Museum of Natural History, 53*(1), 3–308. https://doi.org/10.3374/014.053.0101

Gayon, J. (2000). History of the Concept of Allometry. *American Zoologist, 40*(5), 748–758. https://doi.org/10.1093/icb/40.5.748

Gentilli, A., Cardini, A., Fontaneto, D., & Zuffi, M. A. L. (2009). The phylogenetic signal in cranial morphology of *Vipera aspis*: A contribution from geometric morphometrics. *Herpetological Journal, 19*(2), 69–77.

Gill, T. (1911). A new translation of Aristotle's "History of Animals". *Science, 33*(854), 730-738.

Godefroit, P., Demuynck, H., Dyke, G., Hu, D., Escuillié, F., & Claeys, P. (2013). Reduced plumage and flight ability of a new Jurassic paravian theropod from China. *Nature Communications, 4*, 6–11. https://doi.org/10.1038/ncomms2389

Goldschmidt, R. (1960). *The Material Basis of Evolution*. Pageant Book, Inc.

Gomez, C., Özbudak, E. M., Wunderlich, J., Baumann, D., Lewis, J., & Pourquié, O. (2008). Control of segment number in vertebrate embryos. *Nature, 454*(7202), 335–339. https://doi.org/10.1038/nature07020

Goodwin, B. C. (1982). Development and evolution. *Journal of Theoretical Biology, 97*(1), 43–55. https://doi.org/10.1016/0022-5193(82)90275-2

Gould, S. J. (1966). Allometry and size in ontogeny and phylogeny. *Biological Reviews of the Cambridge Philosophical Society, 41*(4), 587–640. https://doi.org/10.1111/j.1469-185x.1966.tb01624.x

Gould, S. J. (1977). *Ontogeny and Phylogeny*. Harvard University Press.

Gould, S. J. (1996). *The Mismeasure of Man*. W. W. Norton & Company.

Gould, S. J. (2002). *The Structure of Evolutionary Theory*. Harvard University Press.

Gould, S. J., & Eldredge, N. (1977). Punctuated equilibria: The tempo and mode of evolution reconsidered. *Paleobiology, 3*(2), 115-151.

Gould, S. J., & Eldredge, N. (1993). Punctuated equilibrium comes of age. *Nature, 366*, 223–227.

Gould, S. J., & Lewontin, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London - Biological Sciences, 205*(1161), 581–598. https://doi.org/10.1098/rspb.1979.0086

Gould, S. J., & Vrba, E. S. (2013). Exaptation-a missing term in the science of form. *Paleobiology, 8*(1), 4–15.

Gray, J. A., Sherratt, E., Hutchinson, M. N., & Jones, M. E. H. (2019a). Evolution of cranial shape in a continental-scale evolutionary radiation of Australian lizards. *Evolution, 73*(11), 2216–2229. https://doi.org/10.1111/evo.13851

Gray, J. A., Sherratt, E., Hutchinson, M. N., & Jones, M. E. H. (2019b). Changes in ontogenetic patterns facilitate diversification in skull shape of Australian agamid lizards. *BMC Evolutionary Biology, 19*(1). https://doi.org/10.1186/s12862-018-1335-6

Greene, H. W. (1983). Dietary correlates of the origin and radiation of snakes. *Integrative and Comparative Biology, 23*(2), 431–441. https://doi.org/10.1093/icb/23.2.431

Greene, H. W. (1997). *Snakes: The Evolution of Mystery in Nature*. University of California Press.

Greene, H.W., & Cundall, D. (2000). Limbless tetrapods and snakes with legs. *Science, 287*, 1939-1941.

Grizante, M. B., Brandt, R., & Kohlsdorf, T. (2012). Evolution of Body Elongation in

Gymnophthalmid Lizards: Relationships with Climate. *PLoS ONE, 7*(11). https://doi.org/10.1371/journal.pone.0049772

Guerra-fuentes, R. A. (2006). O desenvolvimento embriológico do crânio no clado Iguania e sua contribuição para a filogenia do grupo (Reptilia, Squamata). [Doctoral dissertation, Universidade de São Paulo]. Biblioteca digital USP. https://teses.usp.br/teses/disponiveis/41/41133/tde-26042007-153347/pt-br.php

Guerreiro, I., Nunes, A., Woltering, J. M., Casaca, A., Nóvoa, A., Vinagre, T., Hunter, M. E., Duboule, D., & Mallo, M. (2013). Role of a polymorphism in a *Hox/Pax*-responsive enhancer in the evolution of the vertebrate spine. *Proceedings of the National Academy of Sciences of the United States of America, 110*(26), 10682–10686. https://doi.org/10.1073/pnas.1300592110

Haas, G. (1979). On a new snakelike reptile from the Lower Cenomanian of Ein Jabrud, near Jerusalem. *Bulletin du Muséum national d'Histoire naturelle, Paris (Series 4), 1*,51–64.

Haas, G. (1980a). *Pachyrhachis problematicus* Haas, snakelike reptile from the Lower Cenomanian: ventral view of the skull. *Bulletin du Muséum national d'Histoire naturelle, Paris (Series 4), 2*, 87–104.

Haas, G. (1980b). Remarks on a new ophiomorph reptile from the lower Cenomanian of Ein Jabrud, Israel. In L. L. Jacobs (Ed.), *Aspects of vertebrate history: Essays in Honour of E. H. Colbert* (pp. 177–192). Museum of Northern Arizona Press: Flagstaff AZ.

Haeckel, E. (1866). *Generelle Morphologie der Organismen: allgemeine Grundzuge der organischen Formen-Wissenschaft, mechanisch begrundet durch die von Charles Darwin reformirte Descendenz-Theorie*. G. Reimer, Berlin.

Hall, B. K. (1997). Phylotypic stage or phantom: is there a highly conserved embryonic stage in vertebrates?. *Trends Ecol Evol., 12*, 461-463. 10.1016/S0169-5347(97)01222-6.

Hall, B. K. (1999). *Evolutionary Developmental Biology*. Springer Netherlands.

Hall, B. K. (2013). *Homology: The Hierarchical Basis Of Comparative Biology* (1st Ed.). Academic Press.

Hallgrímsson, B., Jamniczky, H. A., Young, N. M., Rolian, C., Schmidt-Ott, U., & Marcucio, R. S. (2012). The generation of variation and the developmental basis for evolutionary novelty. Journal of Experimental Zoology Part B: *Molecular and Developmental Evolution, 318*(6), 501–517. https://doi.org/10.1002/jez.b.22448

Hallgrímsson, B., Jamniczky, H., Young, N. M., Rolian, C., Parsons, T.E., Boughner, J.C., & Marcucio, R.S. (2009). Deciphering the Palimpsest: Studying the relationship between morphological integration and phenotypic covariation. *Evol Biol., 36*(4), 355–376. doi:10.1007/s11692-009-9076-5.

Halsey, L. G. (2019). The reign of the p-value is over: What alternative analyses could we employ to fill the power vacuum? *Biology Letters, 15*(5). https://doi.org/10.1098/rsbl.2019.0174

Haluska, F., & Alberch, P. (1983). The cranial development of *Elaphe obsoleta* (Ophidia, colubridae). *Journal of Morphology, 178*(1), 37–55. https://doi.org/10.1002/jmor.1051780104

Harari, Y. N. (2015). *Sapiens: A Brief History of Humankind*. Penguin Random House.

Harmon, L. J. , Schulte J. A., Larson, A., & Losos, J. B. (2003). Tempo and mode of evolutionary radiation in iguanian lizards. *Science, 301,*961-964.

Harmon, L. J., Kolbe, J. J., Cheverud, J. M., & Losos, J. B. (2005). Convergence and the multidimensional niche. *Evolution, 59*(2), 409–421. https://doi.org/10.1111/j.0014-3820.2005.tb00999.x

Harmon, L. J., Losos, J. B., Jonathan Davies, T., Gillespie, R. G., Gittleman, J. L., Bryan Jennings, W., Kozak, K. H., McPeek, M. A., Moreno-Roark, F., Near, T. J., Purvis, A., Ricklefs, R. E., Schluter, D., Schulte, J. A., Seehausen, O., Sidlauskas, B. L., Torres-

Carvajal, O., Weir, J. T., & Mooers, A. T. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution, 64*(8), 2385–2396. https://doi.org/10.1111/j.1558-5646.2010.01025.x

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics, 24*(1), 129–131. https://doi.org/10.1093/bioinformatics/btm538

Harrington, S. M., & Reeder, T. W. (2017). Phylogenetic inference and divergence dating of snakes using molecules, morphology and fossils: New insights into convergent evolution of feeding morphology and limb reduction. *Biological Journal of the Linnean Society, 121*(2), 379–394. https://doi.org/10.1093/biolinnean/blw039

Harrington, S. M., Leavitt, D. H., & Reeder, T. W. (2016). Squamate Phylogenetics, Molecular Branch Lengths, and Molecular Apomorphies: A Response to McMahan et al.. *Copeia, 104*(3), 702–707. https://doi.org/10.1643/CG-16-391

Harvey, P. H., & Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford University Press.

Head, J. J., & Polly, P. D. (2015). Evolution of the snake body form reveals homoplasy in amniote *Hox* gene function. *Nature, 520*(7545), 86–89. https://doi.org/10.1038/nature14042

Head, J. J., Bloch, J. I., Hastings, A. K., Bourque, J. R., Cadena, E. A., Herrera, F. A., Polly, P. D., & Jaramillo, C. A. (2009). Giant boid snake from the Palaeocene neotropics reveals hotter past equatorial temperatures. *Nature, 457*(7230), 715–717. https://doi.org/10.1038/nature07671

Hedges, S. B., & Poling, L. L. (1999). A molecular phylogeny of reptiles. *Science, 283*(5404), 998–1001. https://doi.org/10.1126/science.283.5404.998

Heise, P. J., Maxson, L. R., Dowling, H. G., & Hedges, S. B. (1995). Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. *Molecular Biology and Evolution, 12*(2), 259–265. https://doi.org/10.1093/oxfordjournals.molbev.a040202

Helms, J. A., & Schneider, R. A. (2003). Cranial skeletal biology. *Nature, 423*(6937), 326–331. https://doi.org/10.1038/nature01656

Henao-Duque, A. M, & Ceballos, C. P. (2013). Sex-related head size and shape dimorphism in Mapaná snakes (*Bothrops asper*) kept in captivity. *Revista Colombiana de Ciencias Pecuarias*, *26*(3), 201-210.

Hernández-Jaimes, C., Jerez, A., & Ramírez-Pinilla, M. P. (2012). Embryonic development of the skull of the Andean lizard *Ptychoglossus bicolor* (Squamata, Gymnophthalmidae). *Journal of Anatomy, 221*(4), 285–302. https://doi.org/10.1111/j.1469-7580.2012.01549.x

Herrel, A., Schaerlaeken, V., Meyers, J. J., Metzger, K. A., & Ross, C. F. (2007). The evolution of cranial design and performance in squamates: Consequences of skull-bone reduction on feeding behavior. *Integrative and Comparative Biology, 47*(1), 107–117. https://doi.org/10.1093/icb/icm014

Hipsley, C. A., & Müller, J. (2017). Developmental dynamics of ecomorphological convergence in a transcontinental lizard radiation. *Evolution, 71*(4), 936–948. https://doi.org/10.1111/evo.13186

Hipsley, C. A., Rentinck, M. N., Rödel, M. O., & Müller, J. (2016). Ontogenetic allometry constrains cranial shape of the head-first burrowing worm lizard *Cynisca leucura* (Squamata: Amphisbaenidae). *Journal of Morphology, 277*(9), 1159–1167. https://doi.org/10.1002/jmor.20564

Hoffstetter, R. (1960). Un Serpent terrestre dans le Crétacé inférieur du Sahara. *In Bulletin de la Société Géologique de France, 7*(1), 897–902.

Hohl, L. S. L., Barros-Filho, J. D., & Rocha-Barbosa, O. (2018). Skull variation in a shovel-headed amphisbaenian genus, inferred from the geometric morphometric analysis of

five South American *Leposternon* species. *Journal of Morphology*, *279*(11), 1665–1678. https://doi.org/10.1002/jmor.20899

Hollenshead, M. G. (2011). Geometric morphometric analysis of cranial variation in the *Egernia depressa* (Reptilia: Squamata: Scincidae) species complex. *Records of the Western Australian Museum, 26*(2), 138. https://doi.org/10.18195/issn.0312-3162.26(2).2011.138-153

Holman, J. A. (2000). *Fossil Snakes of North America: Origin, Evolution, Distribution, Paleoecology*. Indiana University Press.

Hoso, M., Kameda, Y., Wu, S. P., Asami, T., Kato, M., & Hori, M. (2010). A speciation gene for left-right reversal in snails results in anti-predator adaptation. *Nature Communications, 1*(9). https://doi.org/10.1038/ncomms1133

Houssaye, A. (2010). Rediscovery and description of the second specimen of the hind-limbed snake *Pachyophis woodwardi* Nopcsa, 1923 (Squamata, Ophidia) from the Cenomanian of Bosnia Herzegovina. *Journal of Vertebrate Paleontology, 30*(1), 276–279. https://doi.org/10.1080/02724630903416043

Houssaye, A., Xu, F., Helfen, L., Nil, V. D. E. B., Baumbach, T., & Tafforeau, P. (2011). Three-dimensional pelvis and limb anatomy of the cenomanian hind- limbed snake *Eupodophis descouensi* (Squamata, Ophidia) revealed by synchrotron-radiation computed laminography. *Journal of Vertebrate Paleontology, 31*(1), 2-7.

Hsiang, A. Y., Field, D. J., Webster, T. H., Behlke, A. D. B., Davis, M. B., Racicot, R. A., & Gauthier, J. A. (2015). The origin of snakes: Revealing the ecology, behavior, and evolutionary history of early snakes using genomics, phenomics, and the fossil record. *BMC Evolutionary Biology, 15*(1). https://doi.org/10.1186/s12862-015-0358-5

Hsiou, A. S., Albino, A. M., Medeiros, M. A., & Santos, R. A. B. (2014). The oldest Brazilian snakes from the Cenomanian (Early Late Cretaceous). *Acta Palaeontologica Polonica, 59*(3), 635–642. https://doi.org/10.4202/app.2012.0091

Hugi, J., Mitgutsch, C., & Sánchez-Villagra, M. R. (2010). Chondrogenic and ossification patterns and sequences in White's skink *Liopholis whitii* (Scincidae, Reptilia). *Zoosystematics and Evolution, 86*(1), 21–32. https://doi.org/10.1002/zoos.200900011

Huxley, J. S. (1932). *Problems of Relative Growth*. L. MacVeagh, The Dial Press.

Huxley, J. S., & Teissier, G. (1936). Terminology of relative growth. *Nature, 137*, 780-781.

Huyghe, K., Herrel, A., Adriaens, D., Tadić, Z., & Van Damme, R. (2009). It is all in the head: Morphological basis for differences in bite force among colour morphs of the Dalmatian wall lizard. *Biological Journal of the Linnean Society*, *96*(1), 13–22. https://doi.org/10.1111/j.1095-8312.2008.01103.x

Imhoff, C., Giri, F., Siroski, P., & Amavet, P. (2018). Analysis of morphological variability and heritability in the head of the Argentine Black and White Tegu (*Salvator merianae*): undisturbed vs. disturbed environments. *Zoology*, *127*(February), 47–62. https://doi.org/10.1016/j.zool.2018.02.002

Inoue, J., & Saitou, N. (2020). dbCNS: a new database for conserved noncoding sequences. *Molecular Biology and Evolution*, msaa296, https://doi.org/10.1093/molbev/msaa296

Irie, N., & Kuratani, S. (2011). Comparative transcriptome analysis reveals vertebrate phylotypic period during organogenesis. *Nature Communications, 2*(1). https://doi.org/10.1038/ncomms1248

Irie, N., & Kuratani, S. (2014). The developmental hourglass model: A predictor of the basic body plan? *Development (Cambridge), 141*(24), 4649–4655. https://doi.org/10.1242/dev.107318

Irisarri, I., Baurain, D., Brinkmann, H., Delsuc, F., Sire, J. Y., Kupfer, A., Petersen, J., Jarek, M., Meyer, A., Vences, M., & Philippe, H. (2017). Phylotranscriptomic consolidation of the jawed vertebrate timetree. *Nature Ecology and Evolution, 1*(9),

1370–1378. https://doi.org/10.1038/s41559-017-0240-5

Irish, F. J. (1989). The role of heterochrony in the origin of a novel bauplan: Evolution of the ophidian skull. *Geobios, 22*(2), 227–233. https://doi.org/10.1016/S0016-6995(89)80024-5

Jackson, K. (2002). Post-ovipositional development of the monocled cobra, *Naja kaouthia* (Serpentes: Elapidae). *Zoology, 105*(3), 203–214. https://doi.org/10.1078/0944-2006-00077

Jacob, F. (1977). Evolution and tinkering. *Science, 196*(4295), 1161–1166.

Janensch, W. (1904). Eine fossile Schlange aus dem Eocän des Monte Bolca. Zeitschrift der Deutschen. *Geologischen Gesellschaft, 56*, 54-56.

Janensch, W. (1906). Über *Archaeophis proavus* Mass., eine schlange aus dem Eocän des Monte Bolca. *Beiträge zur Paläontologie und Geologie Oestreich-Ungarns und des Orients, 19*, 1-33.

Jerez, A., Sánchez-Martínez, P. M., & Guerra-Fuentes, R. A. (2015). Embryonic skull development in the neotropical viviparous skink *Mabuya* (Squamata: Scincidae). Desarrollo embrionario del cráneo en la lagartija vivípara *Mabuya* (Squamata: Scincidae). *Acta Zoológica Mexicana (N.S.), 31*(3), 391–402. https://doi.org/10.21829/azm.2015.3131041

Jones, M. E. H., Anderson, C. L., Hipsley, C. A., Müller, J., Evans, S. E., & Schoch, R. R. (2013). Integration of molecules and new fossils supports a Triassic origin for Lepidosauria (lizards, snakes, and tuatara). *BMC Evolutionary Biology, 13*(1). https://doi.org/10.1186/1471-2148-13-208

Kalinka, A. T., Varga, K. M., Gerrard, D. T., Preibisch, S., Corcoran, D. L., Jarrells, J., Ohler, U., Bergman, C. M., & Tomancak, P. (2010). Gene expression divergence recapitulates the developmental hourglass model. *Nature, 468*(7325), 811–816. https://doi.org/10.1038/nature09634

Kaliontzopoulou, A. (2011). Geometric morphometrics in herpetology: modern tools for enhancing the study of morphological variation in amphibians and reptiles. *Basic and Applied Herpetology, 25*, 5–32. https://doi.org/10.11160/bah.11016

Kaliontzopoulou, A., Adams, D. C., van der Meijden, A., Perera, A., & Carretero, M. A. (2012). Relationships between head morphology, bite performance and ecology in two species of *Podarcis* wall lizards. *Evolutionary Ecology*, *26*(4), 825–845. https://doi.org/10.1007/s10682-011-9538-y

Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2007). Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* Lizards. *Journal of Morphology*, 268,152–165. https://doi.org/10.1002/jmor.10494

Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2008). Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: Joining linear and geometric morphometrics. *Biological Journal of the Linnean Society*, 93(1), 111–124. https://doi.org/10.1111/j.1095-8312.2007.00921.x

Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2010). Intraspecific ecomorphological variation: Linear and geometric morphometrics reveal habitat-related patterns within *Podarcis bocagei* wall lizards. *Journal of Evolutionary Biology, 23*(6), 1234–1244. https://doi.org/10.1111/j.1420-9101.2010.01984.x

Kaliontzopoulou, A., Pinho, C., & Martínez-Freiría, F. (2018). Where does diversity come from? Linking geographical patterns of morphological, genetic, and environmental variation in wall lizards. *BMC Evolutionary Biology, 18*(1). https://doi.org/10.1186/s12862-018-1237-7

Kamal, A. M. (1966.) On the process of rotation of the quadrate cartilage in Ophidia. *Anatomischer Anzeiger, 118*(1), 87-90.

Kamal, A. M., & Hammouda, H. G. (1965). The development of the skull of *Psammophis sibilans*. III. The osteocranium of a late embryo. *Journal of Morphology, 116*(2), 297–

310. https://doi.org/10.1002/jmor.1051160207

Kardong, K. V., Kiene, T. L., & Bels, V. (1997). Evolution of trophic systems in squamates. *Netherlands Journal of Zoology, 47*(4), 411–427. https://doi.org/10.1163/156854297x00094

Kazi, S., & Hipsley, C. A. (2018). Conserved evolution of skull shape in Caribbean headfirst burrowing worm lizards (Squamata: Amphisbaenia). *Biological Journal of the Linnean Society, 125*(1), 14–29. https://doi.org/10.1093/BIOLINNEAN/BLY086

Kearney, M., Fujita, M.K., & Ridenour, J. (2009). Lost sex in reptiles: constraints and correlations. In I. Schon, K. Martens, P. van Dijk (Eds.), *Lost Sex* (pp. 447–74). London: Springer

Keates, C., Conradie, W., Greenbaum, E., & Edwards, S. (2019). A snake in the grass: Genetic structuring of the widespread African grass snake (*Psammophylax* Fitzinger 1843), with the description of a new genus and a new species. *Journal of Zoological Systematics and Evolutionary Research*, *57*(4), 1039–1066. https://doi.org/10.1111/jzs.12337

Kendall, D. G. (1977). The diffusion of shape. *Advances in Applied Probability, 9*(03), 428–430. https://doi.org/10.1017/s0001867800028743

Kendall, D. G., Barden, D., Carne, T. K., & Le, H. (1999). *Shape and Shape Theory*. Wiley, Chichester

Khannoon, E. R., & Evans, S. E. (2015). The development of the skull of the Egyptian cobra *Naja h. haje* (Squamata: Serpentes: Elapidae). *PLoS ONE, 10*(4), 1–25. https://doi.org/10.1371/journal.pone.0122185

Khannoon, E. R., & Evans, S. E. (2020). Embryonic skull development in the gecko, *Tarentola annularis* (Squamata: Gekkota: Phyllodactylidae). *Journal of Anatomy, 237*(3), 504–519. https://doi.org/10.1111/joa.13213

Khannoon, E. R., Ollonen, J., & Di-Poï, N. (2020). Embryonic development of skull bones in the Sahara horned viper (*Cerastes cerastes*), with new insights into structures related to the basicranium and braincase roof. *Journal of Anatomy, 237*(1), 1–19. https://doi.org/10.1111/joa.13182

Kikukawa, N., & Hikida, T. (2012). Geometric morphometric analysis of sexual dimorphism and allometric growth in the skull of the Japanese Grass Lizard, *Takydromus tachydromoides* (Reptilia: Lacertidae). *Current Herpetology*, *31*(2), 97–106. https://doi.org/10.5358/hsj.31.97

Klaczko, J., Sherratt, E., & Setz, E. Z. F. (2016). Are diet preferences associated to skulls shape diversification in xenodontine snakes? *PLoS ONE, 11*(2). https://doi.org/10.1371/journal.pone.0148375

Kley, N. J. (2006). Morphology of the lower jaw and suspensorium in the texas blindsnake, *Leptotyphlops dulcis* (Scolecophidia: Leptotyphlopidae). *Journal of Morphology, 267,* 494–515.

Kley, N. J., & Brainerd, E. L. (1999). Feeding by mandibular raking in a snake. *Nature, 402*(6760), 369–370. https://doi.org/10.1038/46460

Klingenberg, C. P. (1998). Heterochrony and allometry: The analysis of evolutionary change in ontogeny. *Biological Reviews, 73*(1), 79–123. https://doi.org/10.1111/j.1469-185X.1997.tb00026.x

Klingenberg, C. P. (2010). Evolution and development of shape: Integrating quantitative approaches. *Nature Reviews Genetics, 11*(9), 623–635. https://doi.org/10.1038/nrg2829

Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources, 11*(2), 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg, C. P. (2013a). Visualizations in geometric morphometrics: How to read and how to make graphs showing shape changes. *Hystrix, 24*(1), 15-24. DOI:

10.4404/hystrix-24.1-7691

Klingenberg, C. P. (2013b). Cranial integration and modularity: Insights into evolution and development from morphometric data. *Hystrix, 24*(1), 43–58. https://doi.org/10.4404/hystrix-24.1-6367

Klingenberg, C. P. (2016). Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution, 226*(3), 113–137. https://doi.org/10.1007/s00427-016-0539-2

Klingenberg, C. P., & Gidaszewski, N. A. (2010). Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Systematic Biology, 59*(3), 245–261. https://doi.org/10.1093/sysbio/syp106

Klingenberg, C. P., & Spence, J. R. (1993). Heterochrony and allometry: Lessons from the water strider genus *Limnoporus*. *Evolution, 47*(6), 1834–1853. https://doi.org/10.1111/j.1558-5646.1993.tb01273.x

Koch, N. M., & Gauthier, J. A. (2018). Noise and biases in genomic data may underlie radically different hypotheses for the position of Iguania within Squamata. *PLoS ONE, 13*(8), 1–29. https://doi.org/10.1371/journal.pone.0202729

Köhler, G., & Haecky, V. (2005). *Incubation of reptile eggs: basics, guidelines, experiences*. Krieger Publishing Company.

Kohlsdorf, T., Grizante, M. B., Navas, C. A., & Herrel, A. (2008). Head shape evolution in Tropidurinae lizards: Does locomotion constrain diet? *Journal of Evolutionary Biology, 21*(3), 781–790. https://doi.org/10.1111/j.1420-9101.2008.01516.x

Kohlsdorf, T., Lynch, V. J., Rodrigues, M. T., Brandley, M. C., & Wagner, G. P. (2010). Data and data interpretation in the study of limb evolution: A reply to Galis et al.. On the reevolution of digits in the lizard genus *Bachia*. *Evolution, 64*(8), 2477–2485. https://doi.org/10.1111/j.1558-5646.2010.01042.x

Kojima, Y., Fukuyama, I., Kurita, T., Hossman, M. Y. Bin, & Nishikawa, K. (2020). Mandibular sawing in a snail-eating snake. *Scientific Reports, 10*(1), 1–5. https://doi.org/10.1038/s41598-020-69436-7

Krell, F. T., & Cranston, P. S. (2004). Which side of the tree is more basal? *Systematic Entomology, 29*(3), 279–281. https://doi.org/10.1111/j.0307-6970.2004.00262.x

Kuhn, T. S. (1970). *The structure of scientific revolutions* (2nd ed.). University of Chicago.

Kumazawa, Y. (2007). Mitochondrial genomes from major lizard families suggest their phylogenetic relationships and ancient radiations. *Gene, 388*(1–2), 19–26. https://doi.org/10.1016/j.gene.2006.09.026

Kvon, E. Z., Kamneva, O. K., Melo, U. S., Barozzi, I., Osterwalder, M., Mannion, B. J., Tissières, V., Pickle, C. S., Plajzer-Frick, I., Lee, E. A., Kato, M., Garvin, T. H., Akiyama, J. A., Afzal, V., Lopez-Rios, J., Rubin, E. M., Dickel, D. E., Pennacchio, L. A., & Visel, A. (2016). Progressive loss of function in a limb enhancer during snake evolution. *Cell, 167*(3), 633-642.e11. https://doi.org/10.1016/j.cell.2016.09.028

Laduke, T. C., Krause, D. W., Scanlon, J. D., & Kley, N. J. (2010). A late Cretaceous (Maastrichtian) snake assemblage from the Maevarano Formation, Mahajanga Basin, Madagascar. *Journal of Vertebrate Paleontology, 30*(1), 109–138. https://doi.org/10.1080/02724630903409188

Lamb, W. R. M. (1925). *Plato in Twelve Volumes* (Vol. 9). Harvard University Press.

Lamborot, M., Vidal, M., Ramírez, C., & Ortiz, J.C. (2005). Intraspecific variation in morphology and sexual dimorphism in *Liolaemus tenuis* (Tropiduridae). Amphibia-Reptilia 26(3): 343-351. https://doi.org/10.1163/156853805774408658

Lazić, M. M., Carretero, M. A., Crnobrnja-Isailović, J., & Kaliontzopoulou, A. (2015). Effects of environmental disturbance on phenotypic variation: An integrated assessment of canalization, developmental stability, modularity, and allometry in lizard head shape. *The American Naturalist, 185*(1), 44–58. https://doi.org/10.1086/679011

Lazić, M. M., Carretero, M. A., Crnobrnja-Isailović, J., & Kaliontzopoulou, A. (2016). Postnatal dynamics of developmental stability and canalization of lizard head shape under different environmental conditions. *Evolutionary Biology, 43*(3), 368–379. https://doi.org/10.1007/s11692-016-9377-4

Lazić, M. M., Rödder, D., & Kaliontzopoulou, A. (2017). The ontogeny of developmental buffering in lizard head shape. *Evolution and Development, 19*(6), 244–252. https://doi.org/10.1111/ede.12238

Leaché, A. D., Koo, M. S., Spencer, C. L., Papenfuss, T. J., Fisher, R. N., & McGuire, J. A. (2009). Quantifying ecological, morphological, and genetic variation to delimit species in the coast horned lizard species complex (*Phrynosoma*). *Proceedings of the National Academy of Sciences of the United States of America*, *106*(30), 12418–12423. https://doi.org/10.1073/pnas.0906380106

Leal, F., & Cohn, M. J. (2016). Loss and re-emergence of legs in snakes by modular evolution of *Sonic hedgeho*g and *HOXD* enhancers. *Current Biology, 26*(21), 2966–2973. https://doi.org/10.1016/j.cub.2016.09.020

Lee, M. S. Y. (1997). The phylogeny of varanoid lizards and the affinities of snakes. *Philosophical Transactions of the Royal Society B: Biological Sciences, 352*(1349), 53–91. https://doi.org/10.1098/rstb.1997.0005

Lee, M. S. Y. (1998). Convergent evolution and character correlation in burrowing reptiles: towards a resolution of squamate relationships. *Biological Journal of Linnean Society, 65,* 369-453.

Lee, M. S. Y. (2000). Soft anatomy, diffuse homoplasy, and the relationships of lizards and snakes. *Zoologica Scripta, 29*, 101–130.

Lee, M. S. Y. (2005a). Molecular evidence and marine snake origins. *Biology Letters, 1*(2), 227–230. https://doi.org/10.1098/rsbl.2004.0282

Lee, M. S. Y. (2005b). Squamate phylogeny, taxon sampling, and data congruence. *Organisms Diversity and Evolution, 5*(1), 25–45. https://doi.org/10.1016/j.ode.2004.05.003

Lee, M. S. Y. (2009). Hidden support from unpromising data sets strongly unites snakes with anguimorph "lizards." *Journal of Evolutionary Biology, 22*(6), 1308–1316. https://doi.org/10.1111/j.1420-9101.2009.01751.x

Lee, M. S. Y., & Caldwell, M. W. (1998). Anatomy and relationships of *Pachyrhachis problematicus*, a primitive snake with hindlimbs. *Philosophical Transactions of the Royal Society B: Biological Sciences, 353*(1375), 1521–1552. https://doi.org/10.1098/rstb.1998.0308

Lee, M. S. Y., & Caldwell, M. W. (2000). Adriosaurus and the affinities of mosasaurs, dolichosaurs, and snakes. *Journal of Paleontology, 74*(5):915-937. DOI: 10.1666/0022-3360(2000)074<0915:AATAOM>2.0.CO;2

Lee, M. S. Y., & Palci, A. (2015). Morphological phylogenetics in the genomic age. *Current Biology, 25*(19), R922–R929. https://doi.org/10.1016/j.cub.2015.07.009

Lee, M. S. Y., & Scanlon, J. D. (2002a). Snake phylogeny based on osteology, soft anatomy and ecology. *Biological Reviews of the Cambridge Philosophical Society, 77*(3), 333–401. https://doi.org/10.1017/S1464793102005924

Lee, M. S. Y., & Scanlon, J. D. (2002b). The Cretaceous marine squamate *Mesoleptos* and the origin of snakes. *Bulletin of The Natural History Museum. Zoology Series, 68*(02). https://doi.org/10.1017/s0968047002000158

Lee, M. S. Y., Bell, G. L., & Caldwell, M. W. (1999a). The origin of snake feeding. *Nature, 400*(6745), 655–658. https://doi.org/10.1038/23236

Lee, M. S. Y., Caldwell, M. W., & Scanlon, J. D. (1999b). A second primitive marine snake: *Pachyophis woodwardi* from the Cretaceous of Bosnia-Herzegovina. *J. Zool., Lond., 248*, 509-520.

Lee, M. S. Y., Hugall, A. F., Lawson, R., & Scanlon, J. D. (2007). Phylogeny of snakes

(Serpentes): Combining morphological and molecular data in likelihood, Bayesian and parsimony analyses. *Systematics and Biodiversity, 5*(4), 371–389. https://doi.org/10.1017/S1477200007002290

Lee, M. S. Y., Palci, A., Jones, M. E. H., Caldwell, M. W., Holmes, J. D., & Reisz, R. R. (2016). Aquatic adaptations in the four limbs of the snake-like reptile *Tetrapodophis* from the Lower Cretaceous of Brazil. *Cretaceous Research, 66*, 194–199. https://doi.org/10.1016/j.cretres.2016.06.004

Lima, F. C. (2015). Ontogenia de *Iguana iguana* (Linnaeus , 1758): estágios embrionários e desenvolvimento do esqueleto [Doctoral dissertation, Universidade de Brasília]. Repositório UnB. http://repositorio.unb.br/bitstream/10482/18640/1/2015_FabianoCamposLima.pdf

Ljubisavljević, K., Polović, L., Urošević, A., & Ivanović, A. (2011). Patterns of morphological variation in the skull and cephalic scales of the lacertid lizard *Algyroides nigropunctatus*. *Herpetological Journal,* 21, 65–72.

Ljubisavljević, K., Urošević, A., Aleksića, I., & Ivanović, A. (2010). Sexual dimorphism of skull shape in a lacertid lizard species *(Podarcis spp., Dalmatolacerta sp., Dinarolacerta sp.)* revealed by geometric morphometrics. *Zoology, 13(3), 168-174.* https://doi.org/10.1016/j.zool.2009.09.003

Lobo, F., Abdala, F., & Scrocchi, G. (1995). Desarrollo del esqueleto de *Liolaemus scapularis* (Iguania: Tropiduridae). *Bollettino Del Museo Regionali Di Scienza Naturali Di Torino, 13*(1), 77–104. http://www.unsa.edu.ar/acunsa/lifetables.pdf

Longrich, N. R., Bhullar, B. A. S., & Gauthier, J. A. (2012). A transitional snake from the Late Cretaceous period of North America. *Nature, 488*(7410), 205–208. https://doi.org/10.1038/nature11227

Losos, J. B. (1999). Uncertainty in the reconstruction of ancestral character states and limitations on the use of phylogenetic comparative methods. *Animal Behaviour*, *58*(6), 1319–1324. https://doi.org/10.1006/anbe.1999.1261

Losos, J. B. (2009). *Lizards in an Evolutionary Tree. Ecology and Adaptive Radiation of Anoles*. University of California Press.

Losos, J. B. (2010). Adaptive radiation, ecological opportunity, and evolutionary determinism *American Naturalist, 175*(6), 623–639. https://doi.org/10.1086/652433

Losos, J. B. (2011). Convergence, adaptation, and constraint. *Evolution, 65*(7), 1827–1840. https://doi.org/10.1111/j.1558-5646.2011.01289.x

Losos, J. B., Hillis, D. M., & Greene. H. W. (2012). Who speaks with a forked tongue? *Science, 338*, 1428-1429.

Lovejoy, A. O. (1976). *The Great Chain of Being: A Study of the History of an Idea*. Harvard University Press.

Lynch, V. J. (2009). Live-birth in vipers (Viperidae) is a key innovation and adaptation to global cooling during the cenozoic. *Evolution, 63*(9), 2457–2465. https://doi.org/10.1111/j.1558-5646.2009.00733.x

Macaluso, L., Carnevale, G., Casu, R., Pietrocola, D., Villa, A., & Delfino, M. (2019). Structural and environmental constraints on reduction of paired appendages among vertebrates. *Biological Journal of the Linnean Society, 128*(2), 473–485. https://doi.org/10.1093/biolinnean/blz097

Macey, J. R., & Verma, A. (1997). Re: Homology in phylogenetic analysis: alignment of transfer RNA genes and the phylogenetic position of snakes. *Molecular Phylogenetics and Evolution, 7*(2), 272–279. https://doi.org/10.1006/mpev.1997.0379

MacFadden, B. J. (2005). Fossil horses - Evidence for evolution. *Science, 307*(5716), 1728–1730. https://doi.org/10.1126/science.1105458

MacLeod, N. (2017). Morphometrics: history, development methods and prospects. *Zoological Systematics, 42*(1), 4–33. https://doi.org/10.11865/zs.201702

Macrì, S., Savriama, Y., Khan, I., & Di-Poï, N. (2019). Comparative analysis of squamate

brains unveils multi-level variation in cerebellar architecture associated with locomotor specialization. *Nature Communications, 10,* 5560 | https://doi.org/10.1038/s41467-019-13405-w

Maddin, H. C., Piekarski, N., Sefton, E. M., & Hanken, J. (2016). Homology of the cranial vault in birds: New insights based on embryonic fate-mapping and character analysis. *Royal Society Open Science, 3*(8). https://doi.org/10.1098/rsos.160356

Maddison, W. P. (1991). Squared-Change Parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Biology, 40*(3), 304–314. https://doi.org/10.1093/sysbio/40.3.304

Mahendra, B. C. (1938). Some remarks on the phylogeny of the Ophidia. *Anat. Anz., 86*, 347-356.

Mahler, D. L., Ingram, T., Revell, L. J., & Losos, J. B. (2013). Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science, 341*(6143), 292–295. https://doi.org/10.1126/science.1232392

Maisano, J. A. (2001). A survey of state of ossification in neonatal squamates. *Herpetol. Monogr., 15,* 135-157.

Maisano, J. A., & Rieppel, O. (2007). The skull of the round island boa, *Casarea dussumieri* Schlegel, based on high-resolution X-Ray computed tomography. *Journal of Morphology, 268,* 371–384.

Mangiacotti, M., Limongi, L., Sannolo, M., Sacchi, R., Zuffi, M. A. L., & Scali, S. (2014). Head shape variation in eastern and western Montpellier snakes. *Acta Herpetologica,* 9(2), 167-177. htttps://doi.org/10.13128/Acta_Herpetol-14194

Manier, M. K. (2004). Geographic variation in the long-nosed snake, *Rhinocheilus lecontei* (Colubridae): Beyond the subspecies debate. *Biological Journal of the Linnean Society*, *83*(1), 65–85. https://doi.org/10.1111/j.1095-8312.2004.00373.x

Margolis, E., & Laurence, S. (2007). The ontology of concepts - abstract objects or mental representations? *Nous, 41*(4), 561–593. https://doi.org/10.1111/j.1468-0068.2007.00663.x

Martill, D. M., Tischlinger, H., & Longrich, N. R. (2015). A four-legged snake from the Early Cretaceous of Gondwana. *Science, 349*(6246), 416–419. https://doi.org/10.1126/science.aaa9208

Massalongo, D. A. B. (1859). *Specimen photographicum animalium quorundam plantarumque fossilium agri Veronensis*. Vicentini-Franchini.

Massetti, F., Kaliontzopoulou, A., Gomes, V., & Rato, C. (2019). Variation in morphology and functional performance across distinct evolutionary lineages of the Moorish gecko (*Tarentola mauritanica*) from the Iberian Peninsula. *Journal of Zoological Systematics and Evolutionary Research, 57*(2), 431–444. https://doi.org/10.1111/jzs.12249

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., & Wolpert, L. (1985). Developmental constraints and evolution: A perspective from the mountain lake conference on development and evolution. *The Quarterly Review of Biology, 60*(3), 265-287.

Mayr, E. (1960). The emergence of novelty. In S. Tax (ed.), *The Evolution of Life,* (pp. 349–380). University of Chicago Press.

Mayr, E. (1963). *Animal Species and Evolution*. Harvard University Press.

Mayr, E. (1982). *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*. Harvard University Press.

McCartney, J. A., & Seiffert, E. R. (2016). A Late Eocene snake fauna from the Fayum Depression, Egypt. *Journal of Vertebrate Paleontology, 36*(1). https://doi.org/10.1080/02724634.2015.1029580

McCurry, M. R., Mahony, M., Clausen, P. D., Quayle, M. R., Walmsley, C. W., Jessop, T. S., Wroe, S., Richards, H., & McHenry, C. R. (2015). The relationship between cranial

structure, biomechanical performance and ecological diversity in varanoid lizards. *PLoS ONE, 10*(6), 1–27. https://doi.org/10.1371/journal.pone.0130625

Mcdowell, S. B. (1972). The evolution of the tongue of snakes, and its bearing on snake origins. In *T.* Dobzhansky, M. K. Hecht, W. C. Steere (Eds.), *Evolutionary Biology* (Volume 6, pp 191–273). Appleton-Century-Crofts.

McDowell, S. B. (2008). The skull of serpentes. In C. Gans, A. S. Gaunt and K. Adler (Eds.), *Biology of the Reptilia* (Volume 21, Morphology I, pp. 467-620). Society of the study of Amphibians and Reptiles.

McDowell, S. B., & Bogert, C. M. (1954). The systematic position of *Lanthanotus* and the affinities of the anguinomorphan lizards. *Bulletin of the American Museum of Natural History, 105*, 1–142. http://digitallibrary.amnh.org/handle/2246/1146

Mckinney, M., & McNamara, K. J. (1991). *Heterochrony the Evolution of Ontogeny*. Springer US.

McNamara, K. J. (1986). A guide to the nomenclature of heterochrony. *Journal of Paleontology, 60*(1), 4-13

Mcnamara, K. J., & Mckinney, M. L. (2005). Heterochrony , disparity , and macroevolution. *Paleobiology, 31*, 17-26. https://doi.org/10.1666/0094-8373(2005)031

Metzger, K. A., & Herrel, A. (2005). Correlations between lizard cranial shape and diet: A quantitative, phylogenetically informed analysis. *Biological Journal of the Linnean Society, 86*(4), 433–466. https://doi.org/10.1111/j.1095-8312.2005.00546.x

Miralles, A., Marin, J., Markus, D., Herrel, A., Hedges, S. B., & Vidal, N. (2018). Molecular evidence for the paraphyly of Scolecophidia and its evolutionary implications. *Journal of Evolutionary Biology, 31*(12), 1782–1793. https://doi.org/10.1111/jeb.13373

Mitteroecker, P., & Huttegger, S. M. (2009). The concept of morphospaces in evolutionary and developmental biology: mathematics and metaphors. *Biological Theory, 4*(1), 54–67. https://doi.org/10.1162/biot.2009.4.1.54

Mitteroecker, P., Gunz, P., & Bookstein, F. L. (2005). Heterochrony and geometric morphometrics: A comparison of cranial growth in *Pan paniscus* versus *Pan troglodytes*. *Evolution and Development, 7*(3), 244–258. https://doi.org/10.1111/j.1525-142X.2005.05027.x

Mitteroecker, P., Gunz, P., Bernhard, M., Schaefer, K., & Bookstein, F. L. (2004). Comparison of cranial ontogenetic trajectories among great apes and humans. *Journal of Human Evolution, 46*(6), 679–698. https://doi.org/10.1016/j.jhevol.2004.03.006

Moczek, A. P. (2008). On the origins of novelty in development and evolution. *BioEssays, 30*(5), 432–447. https://doi.org/10.1002/bies.20754

Monteiro, L. R. (1999). Points of view multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *System. Biol., 48*(1), 192-199.

Monteiro, L. R. (2013). Morphometrics and the comparative method: Studying the evolution of biological shape. *Hystrix, 24*(1), 25–32. https://doi.org/10.4404/hystrix-24.1-6282

Monteiro, L. R., & Abe, A. S. (1997). Allometry and morphological integration in the skull of *Tupinambis merianae* (Lacertilia: Teiidae). *Amphibia Reptilia, 18*(4), 397–405. https://doi.org/10.1163/156853897X00440

Moon, B. R., Penning, D. A., Segall, M., & Herrel, A. (2019). Feeding in snakes: form, function, and evolution of the feeding system (pp. 527–574). In: V. Bels & I. Q. Whishaw (eds.), *Feeding in Vertebrates*. Springer, cham. https://doi.org/10.1007/978-3-030-13739-7_14

Moshtaghie, M., Kaboli, M., & Salehi, M. (2018). Geometric morphometric analysis of head shape in a spotted whip snake (*Hemorrhois ravergieri*) in different habitats of Iran. *Acta Medica Mediterranea*, *34*, 583. https://doi.org/10.19193/0393-

6384_2018_2s_92

Mulcahy, D. G., Noonan, B. P., Moss, T., Townsend, T. M., Reeder, T. W., Sites, J. W., & Wiens, J. J. (2012). Estimating divergence dates and evaluating dating methods using phylogenomic and mitochondrial data in squamate reptiles. *Molecular Phylogenetics and Evolution, 65*(3), 974–991. https://doi.org/10.1016/j.ympev.2012.08.018

Müller, G. B. (2010). Epigenetic innovation. In M. Pigliucci and G.B. Müller (Eds.), *Evolution - The Extended Synthesis* (pp. 307–333). MIT Press.

Müller, J., Hipsley, C. A., Head, J. J., Kardjilov, N., Hilger, A., Wuttke, M., & Reisz, R. R. (2011). Eocene lizard from Germany reveals amphisbaenian origins. *Nature, 473*(7347), 364–367. https://doi.org/10.1038/nature09919

Müller, J., Scheyer, T. M., Head, J. J., Barrett, P. M., Werneburg, I., Ericson, P. G. P., Pol, D., & Sánchez-Villagra, M. R. (2010). Homeotic effects, somitogenesis and the evolution of vertebral numbers in recent and fossil amniotes. *Proceedings of the National Academy of Sciences of the United States of America, 107*(5), 2118–2123. https://doi.org/10.1073/pnas.0912622107

Murta-Fonseca, R. A., & Fernandes, D. S. (2016). The skull of *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854) (Serpentes: Dipsadidae) as a model of snake ontogenetic allometry inferred by geometric morphometrics. *Zoomorphology*, *135*(2), 233–241. https://doi.org/10.1007/s00435-015-0297-0

Murta-Fonseca, R. A., Machado, A., Lopes, R. T., Fernandes, D. S. (2019). Sexual dimorphism in *Xenodon neuwiedii* skull revealed by geometric morphometrics (Serpentes; Dipsadidae). *Amphibia Reptilia*, *April*. https://doi.org/10.1163/15685381-20191147

Nicula, B. -I. (2020). Cracking the Snake Detection Theory: The subcortical visual pathway as a major player in cultural transformations. *World Journal of Neuroscience, 10*(04), 166–190. https://doi.org/10.4236/wjns.2020.104018

Niedźwiedzki, G., Szrek, P., Narkiewicz, K., Narkiewicz, M., & Ahlberg, P. E. Tetrapod trackways from the early Middle Devonian period of Poland. *Nature, 463*, 43-48.

Nopcsa, F. (1923). *Eidolosaurus* und *Pachyophis*. Zwei neue Neocom-Reptilien. *Palaeontographica, 65*, 97–154.

Nopsca, F. (1908). Zur kenntnis der fossilen Eidechsen. Beitr. *PalaXont. geol. Orsterreih-Ungarns*, 21, 33-62.

Oakley, T. H., & Cunningham, C. W. (2000). Independent contrasts succeed where ancestor reconstruction fails in a known bacteriophage phylogeny. *Evolution*, *54*(2), 397–405. https://doi.org/10.1111/j.0014-3820.2000.tb00042.x

Oguz, I., Cates, J., Datar, M., Paniagua, B., Fletcher, T., Vachet, C., Styner, M., & Whitaker, R. (2016). Entropy-based particle correspondence for shape populations. *International Journal of Computer Assisted Radiology and Surgery, 11*(7), 1221–1232. https://doi.org/10.1007/s11548-015-1319-6

Olori, J. C., & Bell, C. J. (2012). Comparative skull morphology of uropeltid snakes (Alethinophidia: Uropeltidae) with special reference to disarticulated elements and variation. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0032450

Olson, E. C. (1981). The problem of missing links: today and yesterday. *The Quarterly Review of Biology, 56*(4), 405-442.

Olson, E. C., & Miller R.L. (1958). *Morphological Integration*. University of Chicago Press.

Omland, K. E., Cook, L. G., & Crisp, M. D. (2008). Tree thinking for all biology: The problem with reading phylogenies as ladders of progress. *BioEssays, 30*(9), 854–867. https://doi.org/10.1002/bies.20794

Openshaw, G. H., & Keogh, J. S. (2014). Head shape evolution in monitor lizards (*Varanus*): Interactions between extreme size disparity, phylogeny and ecology. *Journal of Evolutionary Biology, 27*(2), 363–373. https://doi.org/10.1111/jeb.12299

Openshaw, G. H., D'Amore, D. C., Vidal-García, M., & Keogh, J. S. (2017). Combining

geometric morphometric analyses of multiple 2D observation views improves interpretation of evolutionary allometry and shape diversification in monitor lizard (*Varanus*) crania. *Biological Journal of the Linnean Society, 120*(3), 539–552. https://doi.org/10.1111/bij.12899

Oppel, M. (1811). *Die Ordnungen, Familien, und Gattungen der Reptilien, als Prodrom einer Naturgeschichte derselben*. Munich: Joseph Lindauer.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P. L. F., Fumagalli, M., Vilstrup, J. T., Raghavan, M., Korneliussen, T., Malaspinas, A. S., Vogt, J., Szklarczyk, D., Kelstrup, C. D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A.M.V., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G.D., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J. F., Weinstock, J., Gregersen, K., Røed, K.H., Eisenmann, V., Rubin, C.J., Miller, D.C., Antczak, D. F., Bertelsen, M.F., Brunak, S., Al-Rasheid, K.A.S., Ryder, O., Andersson, L., Mundy, J., Krogh, A., Gilbert, M. T. P., Kjær, K., Sicheritz-Ponten, T., Jensen, L. J., Olsen, J. V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., & Willerslev, E. (2013). Recalibrating equus evolution using the genome sequence of an Early Middle Pleistocene horse. *Nature, 499*(7456), 74–78. https://doi.org/10.1038/nature12323

Osborn, H. F. (1902). The Law of Adaptive Radiation. *The American Naturalist, 36*(425), 353-363.

Owen, R. (1877). On the rank and affinities in the reptilian class of the Mosasauridae, Gervais. *Q. Jl. geol. Soc., 34,* 748-753.

Oxnard, C., Lestrel, P. E., & Oxnard, C. E. (Ed.) (2000). *Morphometrics for the Life Sciences*. World Scientific Publishing.

Palci, A., Caldwell, M. W., & Albino A. M. (2013a). Emended diagnosis and phylogenetic relationships of the upper cretaceous fossil snake *Najash rionegrina* Apesteguía and Zaher, 2006. *Journal of Vertebrate Paleontology,33*(1), 131–140.

Palci, A. (2014). On the Origin and Evolution of the Ophidia. [Doctoral dissertation, University of Alberta]. Era Library University of Alberta. https://era.library.ualberta.ca/items/08055594-6458-4185-a058-0dfe62841f24

Palci, A., & Caldwell, M. W. (2014). The upper cretaceous snake *Dinilysia patagonica* Smith-Woodward, 1901, and the crista circumfenestralis of snakes. *Journal of Morphology, 275*(10), 1187–1200. https://doi.org/10.1002/jmor.20297

Palci, A., & Lee, M. S. Y. (2018). Geometric morphometrics, homology and cladistics: review and recommendations. *Cladistics, 35*(2), 230–242. https://doi.org/10.1111/cla.12340

Palci, A., Caldwell, M. W., & Nydam, R. L. (2013b). Reevaluation of the anatomy of the Cenomanian (Upper Cretaceous) hind-limbed marine fossil snakes *Pachyrhachis, Haasiophis,* and *Eupodophis*. *Journal of Vertebrate Paleontology, 33*(6), 1328–1342. https://doi.org/10.1080/02724634.2013.779880

Palci, A., Hutchinson, M. N., Caldwell, M. W., & Lee, M. S. Y. (2017). The morphology of the inner ear of squamate reptiles and its bearing on the origin of snakes. *Royal Society Open Science, 4*(8). https://doi.org/10.1098/rsos.170685

Palci, A., Hutchinson, M. N., Caldwell, M. W., Scanlon, J. D., & Lee, M. S. Y. (2018). Palaeoecological inferences for the fossil Australian snakes *Yurlunggur* and *Wonambi* (Serpentes, madtsoiidae). *Royal Society Open Science, 5*(3). https://doi.org/10.1098/rsos.172012

Palci, A., Lee, M. S. Y., & Hutchinson, M. N. (2016). Patterns of postnatal ontogeny of the skull and lower jaw of snakes as revealed by micro-CT scan data and three-dimensional geometric morphometrics. *Journal of Anatomy, 229*(6), 723–754. https://doi.org/10.1111/joa.12509

Peterson, T., & Müller, G. B. (2013). What is evolutionary novelty? Process versus character based definitions. *Journal of Experimental Zoology Part B: Molecular and*

Developmental Evolution, 320(6), 345–350. https://doi.org/10.1002/jez.b.22508

Peyer, B. (1912). Die Entwicklung des Schädelskelettes von *Vipera aspis*. *Morphologisches Jahrbuch, 44*(4), 563-621.

Pianka, E. R., & Vitt, L. J. (2003). *Lizards: Windows to the Evolution of Diversity*. University of California Press.

Piekarski, N., Gross, J. B., & Hanken, J. (2014). Evolutionary innovation and conservation in the embryonic derivation of the vertebrate skull. *Nature Communications, 5*, 1–9. https://doi.org/10.1038/ncomms6661

Pigliucci, M. (2003). Phenotypic integration: Studying the ecology and evolution of complex phenotypes. *Ecology Letters, 6*(3), 265–272. https://doi.org/10.1046/j.1461-0248.2003.00428.x

Pigliucci, M. (2008). What, if anything, is an evolutionary novelty? *Philosophy of Science, 75*(5), 887–898. https://doi.org/10.1086/594532

Piras, P., Salvi, D., Ferrara, G., Maiorino, L., Delfino, M., Pedde, L., & Kotsakis, T. (2011). The role of post-natal ontogeny in the evolution of phenotypic diversity in *Podarcis* lizards. *Journal of Evolutionary Biology, 24*(12), 2705–2720. https://doi.org/10.1111/j.1420-9101.2011.02396.x

Polachowski, K. M., & Werneburg, I. (2013). Late embryos and bony skull development in *Bothropoides jararaca* (Serpentes, Viperidae). *Zoology, 116*(1), 36–63. https://doi.org/10.1016/j.zool.2012.07.003

Polcyn, M. J., Jacobs, L. L., & Haber, A. (2005). A morphological model and CT assessment of the skull of *Pachyrhachis problematicus* (squamata, serpentes), a 98 million year old snake with legs from the Middle East. *Palaeontologia Electronica, 8*(1).

Polly, P. D. (2001). Paleontology and the comparative method: Ancestral node reconstructions versus observed node values. *American Naturalist*, *157*(6), 596–609. https://doi.org/10.1086/320622

Polly, P. D. (2013). Evolution: Stuck between the teeth. *Nature, 497*(7449), 325–326. https://doi.org/10.1038/nature12099

Polly, P. D. (2020). Functional tradeoffs carry phenotypes across the valley of the shadow of death. *Integrative and Comparative Biology*, 60(5),1268–1282.

Pomidor, B. J., Makedonska, J., & Slice, D. E. (2016) A landmark-free method for three-dimensional shape analysis. *PLoS ONE, 11*(3): e0150368. https://doi.org/10.1371/journal.pone.0150368

Pontoppidan, E. (1755). *The natural history of Norway: containing a particular and accurate account of the temperature of the air,the different soils, waters, vegetables, metals, minerals, stones, beasts, birds, and fishes : together with the dispositions, customs, and manner of living of the inhabitants : interspersed with physiological notes from eminent writers, and transactions of academics : in two parts*. London, printed for A. Linde.

Popper, K. (1959). *The Logic of Scientific Discovery*. Basic Books.

Posner, G. J., Strike, K. A., Hewson, P. W., & Gertzog, W. A. (1982). Accommodation of a scientific conception: Toward a theory of conceptual change. *Science Education, 66*(2), 211–227. https://doi.org/10.1002/sce.3730660207

Pough, F. H., R. M. Andrews, J. E. Cadle, M. L. Crump, A. H. Savitsky, & K. D.Wells. (2004). *Herpetology* (3rd ed.). Prentice Hall, Upper Saddle River, New Jersey.

Powell, G. L., Russell, A. P., Jamniczky, H. A., & Hallgrímsson, B. (2017). Shape variation in the dermatocranium of the greater short-horned lizard *Phrynosoma hernandesi* (Reptilia: Squamata: Phrynosomatidae). *Evolutionary Biology, 44*(2), 240–260. https://doi.org/10.1007/s11692-016-9403-6

Prothero, D. R., & Buell, C.D. (2007). *Evolution: What the Fossils Say and Why it Matters*. Columbia University Press.

Pyron, R. A. (2017). Novel approaches for phylogenetic inference from morphological data and total-evidence dating in squamate reptiles (Lizards, Snakes, and Amphisbaenians). *Systematic Biology, 66*(1), 38–56. https://doi.org/10.1093/sysbio/syw068

Pyron, R. A., & Burbrink, F. T. (2014). Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. *Ecology Letters, 17*(1), 13–21. https://doi.org/10.1111/ele.12168

Pyron, R. A., Burbrink, F. T., & Wiens, J. J. (2013). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology, 13*(1), 1. https://doi.org/10.1186/1471-2148-13-93

Raff, R. (1996). *The Shape of Life: Genes, Development, and the Evolution of Animal Form*. University of Chicago Press, Chicago.

Rage, J-C., & Escuillié, F. (2000). Un nouveau serpent bipède du Cénomanien (Crétacé). Implications phylétiques. *Acad. Sci. Paris, Sciences de la Terre et des planètes / Earth and Planetary Sciences, 330*, 513–520.

Rage, J. -C., & Escuillié, F. (2002). *Eupodophis*, new name for the genus *Podophis* Rage and Escuillié, 2000, an extinct bipedal snake, preoccupied by *Podophis* Wiegmann, 1834 (Lacertilia, Scincidae). *Amphibia-Reptilia, 23*, 232–233.

Rage, J. -C., & Escuillié, F. (2003). The Cenomanian: stage of hindlimbed snakes. *Carnets de Géologie (Notebooks on Geology), Maintenon, Article 2003/01* (CG2003_A01). https://doi.org/10.4267/2042/293

Rage, J. -C., & Werner, C. (1999). Mid-cretaceous (Cenomanian) snakes from Wadi Abu Hashim, Sudan: the earliest snake assemblage. *Palaeont. Afr., 35*, 85-110. http://wiredspace.wits.ac.za/handle/10539/16454

Rage, J. -C., Vullo, R., & Néraudeau, D. (2016). The mid-Cretaceous snake *Simoliophis rochebrunei* Sauvage, 1880 (Squamata: Ophidia) from its type area (Charentes, southwestern France): Redescription, distribution, and palaeoecology. *Cretaceous Research, 58*, 234–253. https://doi.org/10.1016/j.cretres.2015.10.010

Rage, J.-C. (1984). *Handbuch der PalaÈoherpetologie. Teil 11. Serpentes*. Stuttgart: Gustav Fischer Verlag.

Raia, P., Guarino, F. M., Turano, M., Polese, G., Rippa, D., Carotenuto, F., Monti, D. M., Cardi, M., & Fulgione, D. (2010). The blue lizard spandrel and the island syndrome. *BMC Evol. Biol.,10*, 289. https://doi.org/10.1186/1471-2148-10-289.

Raup, D. M. (1994). The role of extinction in evolution. *Proc. Natl Acad. Sci. USA, 91*, 6758–6763.

Reeder, T. W., Townsend, T. M., Mulcahy, D. G., Noonan, B. P., Wood, P. L., Sites, J. W., & Wiens, J. J. (2015). Integrated analyses resolve conflicts over squamate reptile phylogeny and reveal unexpected placements for fossil taxa. *PLoS ONE, 10*(3), 1–22. https://doi.org/10.1371/journal.pone.0118199

Rest, J. S., Ast, J. C., Austin, C. C., Waddell, P. J., Tibbetts, E. A., Hay, J. M., & Mindell, D. P. (2003). Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. *Molecular Phylogenetics and Evolution*, *29*(2), 289–297. https://doi.org/10.1016/S1055-7903(03)00108-8

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*(2), 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Revell, L. J., Harmon, L. J., & Collar, D. C. (2008). Phylogenetic signal, evolutionary process, and rate. *Systematic Biology*, *57*(4), 591–601. https://doi.org/10.1080/10635150802302427

Reynolds, R. G., Collar, D. C., Pasachnik, S. A., Niemiller, M. L., Puente-Rolón, A. R., & Revell, L. J. (2016). Ecological specialization and morphological diversification in Greater Antillean boas. *International Journal of Organic Evolution*, *70*(8), 1882–1895. https://doi.org/10.1111/evo.12987

Richard, D. (2009). *The Selfish Gene*. Oxford University Press

Richardson, M. K. (1995). Heterochrony and the phylotypic period. *Developmental Biology, 172*(2), 412–421. https://doi.org/10.1006/dbio.1995.8041

Richardson, M. K., Hanken, J., Gooneratne, M. L., Pieau, C., Raynaud, A., Selwood, L., & Wright, G. M. (1997). There is no highly conserved embryonic stage in the vertebrates: Implications for current theories of evolution and development. *Anatomy and Embryology*, *196*(2), 91–106. https://doi.org/10.1007/s004290050082

Ricklefs, R. E., Losos, J. B., & Townsend, T. M. (2007). Evolutionary diversification of clades of squamate reptiles. *Journal of Evolutionary Biology, 20*(5), 1751–1762. https://doi.org/10.1111/j.1420-9101.2007.01388.x

Ridley, M. (2003). *Evolution (3rd Ed.)*. John Wiley and Sons Ltd.

Rieppel, O. (1978). The evolution of the naso-frontal joint in snakes and its bearing on snake origins. *Journal of Zoological Systematics and Evolutionary Research, 16*(1), 14–27. https://doi.org/10.1111/j.1439-0469.1978.tb00917.x

Rieppel, O. (1984). The upper temporal arcade of lizards: an ontogenetic problem. *Rev. Suisse Zool., 91*, 475-482.

Rieppel, O. (1988). A review of the origin of snakes. In M. K. Hecht, B. Wallace, S. G. T. Prance (Eds.), *Evolutionary Biology* (Volume 22, pp 37–130). Plenum Press.

Rieppel, O. (1992a). Studies on skeleton formation in reptiles. I. The postembryonic development of the skeleton in *Cyrtodactylus pubisulcus* (Reptilia: Gekkonidae). *Journal of Zoology, 227*(1), 87–100. https://doi.org/10.1111/j.1469-7998.1992.tb04346.x

Rieppel, O. (1992b). Studies on skeleton formation in reptiles. III, Patterns of ossification in the skeleton of *Lacerta vivipara* Jacquin (Reptilia, Squamata). *Fieldiana - Zoology, 68,* 1-25. https://doi.org/10.5962/bhl.title.2692

Rieppel, O. (1993a). Patterns of diversity in the reptilian skull. In J. Hanken & B.K. Hall (Eds.), *The Skull: Patterns of structural and systematic diversity* (Volume 2, pp. 334-390). The University of Chicago Press, Chicago.

Rieppel, O. (1993b). Studies on skeleton formation in reptiles. II. *Chamaeleo hoehnelii* (Squamata: Chamaeleoninae), with comments on the homology of carpal and tarsal bones. *Herpetologica, 49*(1), 66–78.

Rieppel, O. (1994). Studies on skeleton formation in reptiles. Patterns of ossification in the skeleton of *Lacerta agilis* exigua Eichwald (Reptilia, Squamata). *J. Herpetol. 2*8, 145-153.

Rieppel, O., & Gronowski, R. W. (1981). The loss of the lower temporal arcade in diapsid reptiles. *Zoological Journal of the Linnean Society, 72*(3), 203–217. https://doi.org/10.1111/j.1096-3642.1981.tb01570.x

Rieppel, O., & Head, J. J. (2004). New specimens of the fossil snake genus *Eupodophis* Rage and Escuillié, from Cenomanian (Late Cretaceous) of Lebanon. *Memorie della Societ `a Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano, 32,* 1–26.

Rieppel, O., & Kearney, M. (2001). The origin of snakes: limits of a scientific debate. *Biologist (London), 48*(3):110-114. PMID: 11399841.

Rieppel, O., & Kearney, M. (2002). Similarity. *Biological Journal of the Linnean Society, 75*, 59–82. https://doi.org/10.1046/j.1095-8312.2002.00006.x

Rieppel, O., & Zaher, H. (2000). The intramandibular joint in squamates, and the phylogenetic relationships of the fossil snake *Pachyrhachis problematicus* Haas. *Fieldiana: Geology, 43*, 1-69. https://doi.org/10.5962/bhl.title.3584

Rieppel, O., & Zaher, H. (2001). The development of the skull in *Acrochordus granulatus* (Schneider) (Reptilia: Serpentes), with special consideration of the otico-occipital complex. *Journal of Morphology, 249*(3), 252–266. https://doi.org/10.1002/jmor.1053

Rieppel, O., Kluge, A. G., & Zaher, H. (2002). Testing the phylogenetic relationships of the Pleistocene snake *Wonambi naracoortensis* Smith. *Journal of Vertebrate Paleontology, 22*(4), 812–829. https://doi.org/10.1671/0272-4634(2002)022[0812:TTPROT]2.0.CO;2

Rieppel, O., Zaher, H., Tchernov, E., & Polcyn, M. J. (2003). The anatomy and relationships of *Haasiophis terrasanctus*, a fossil snake with well-developed hind limbs from the Mid-Cretaceous of the Middle East. *Journal of Paleontology, 77*(3), 536–558. https://doi.org/10.1666/0022-3360(2003)077<0536:taaroh>2.0.co;2

Rohlf, F. J. (1990). Morphometrics. *Annu. Rev. Ecol. Syst, 21*, 299–316.

Rohlf, F. J. (1999). Shape statistics: Procrustes superimpositions and tangent spaces. *Journal of Classification, 16*(2), 197–223. https://doi.org/10.1007/s003579900054

Rohlf, F. J. (2002). Geometric morphometrics in phylogeny. In P. Forey & N. Macleod (Eds.). *Morphology, shape and phylogenetics* (pp. 175–193). Taylor & Francis, London.

Rohlf, F. J. (2013). tpsDig v.2.17. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. (2014). tpsSmall ver.1.29. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J., & Marcus, L. F. (1993). A revolution in morphometrics. *Trends in Ecology and Evolution, 8*(4), 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

Rohlf, F. J., & Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology, 39*(1), 40–59. https://doi.org/10.2307/2992207

Ronquist, F. (2004). Bayesian inference of character evolution. *Trends in Ecology and Evolution*, *19*(9), 475–481. https://doi.org/10.1016/j.tree.2004.07.002

Roscito, J. G. (2010). Desenvolvimento embrionário e a evolução da fossorialidade nos lagartos da tribo Gymnophthalmini (Squamata, Gymnophthalmidae). [Doctoral dissertation, Universidade]. Biblioteca Digital USP. https://teses.usp.br/teses/disponiveis/41/41133/tde-02022011-172520/pt-br.php

Roscito, J. G., & Rodrigues, M. T. (2012). Skeletal development in the fossorial gymnophthalmids *Calyptommatus sinebrachiatus* and *Nothobachia ablephara*. *Zoology, 115*(5), 289–301. https://doi.org/10.1016/j.zool.2012.02.004

Roscito, J. G., Sameith, K., Parra, G., Langer, B. E., Petzold, A., Moebius, C., Bickle, M., Rodrigues, M. T., & Hiller, M. (2018). Phenotype loss is associated with widespread divergence of the gene regulatory landscape in evolution. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-07122-z

Ruane, S. (2015). Using geometric morphometrics for integrative taxonomy: An examination of head shapes of milksnakes (genus *Lampropeltis*). *Zoological Journal of the Linnean Society*, *174*(2), 394–413. https://doi.org/10.1111/zoj.12245

Russell, E. S. (1916). *Form and Function: A Contribution to the History of Animal Morphology*. J. Murray.

Sagonas, K., Pafilis, P., Lymberakis, P., Donihue, C. M., Herrel, A., & Valakos, E. D. (2014). Insularity affects head morphology, bite force and diet in a Mediterranean lizard. *Biological Journal of the Linnean Society*, *112*(3), 469–484. https://doi.org/10.1111/bij.12290

Saint, K. M., Austin, C. C., Donnellan, S. C., & Hutchinson, M. N. (1998). C-mos, a nuclear marker useful for squamate phylogenetic analysis. *Molecular Phylogenetics and Evolution*, *10*(2), 259–263. https://doi.org/10.1006/mpev.1998.0515

Salazar-Ciudad, A. I., & Marín-Riera, M. (2013). Adaptive dynamics under development-based genotype- phenotype maps. *Nature, 497*, 361–364.

Salazar-Ciudad, I., & Jernvall, J. (2005). Graduality and innovation in the evolution of complex phenotypes: Insights from development. *Journal of Experimental Zoology*

Part B: Molecular and Developmental Evolution, *304*(6), 619–631. https://doi.org/10.1002/jez.b.21058

Salazar-Ciudad, I., Jernvall, J., & Newman, S. A. (2003). Mechanisms of pattern formation in development and evolution. *Development*, *130*(10), 2027–2037. https://doi.org/10.1242/dev.00425

Salsburg, D. (2001). *The Lady Tasting Tea: How Statistics Revolutionized Science in the Twentieth Century*. New York: W.H. Freeman.

Salvador-Martínez, I. (2016). Estimating complexity and adaptation in the embryo: a statistical developmental biology approach. [Doctoral dissertation, University of Helsinki]. Helda database. https://helda.helsinki.fi/bitstream/handle/10138/169225/estimati.pdf?sequence=1

Sanger, T. J., Mahler, D. L., Abzhanov, A., & Losos, J. B. (2012). Roles for modularity and constraint in the evolution of cranial diversity among *Anolis* lizards. *Evolution, 66*(5), 1525–1542. https://doi.org/10.1111/j.1558-5646.2011.01519.x

Sanger, T. J., Seav, S. M., Tokita, M., Langerhans, R. B., Ross, L. M., Losos, J. B., & Abzhanov, A. (2014). The oestrogen pathway underlies the evolution of exaggerated male cranial shapes in *Anolis* lizards. *Proceedings of the Royal Society B: Biological Sciences, 281*(1784). https://doi.org/10.1098/rspb.2014.0329

Sanger, T. J., Sherratt, E., Mcglothlin, J. W., Brodie, E. D., Losos, J. B., & Abzhanov, A. (2013). Convergent evolution of sexual dimorphism in skull shape using distinct developmental strategies. *Evolution, 67*(8), 2180–2193. https://doi.org/10.1111/evo.12100

Scanferla A. (2016). Postnatal ontogeny and the evolution of macrostomy in snakes.*R. Soc. open sci., 3,* 160612. http://dx.doi.org/10.1098/rsos.160612

Scanferla, A., & Bhullar, B. A. S. (2014). Postnatal development of the skull of *Dinilysia patagonica* (Squamata-stem Serpentes). *Anatomical Record, 297*(3), 560–573. https://doi.org/10.1002/ar.22862

Scanferla, A., & Smith, K. T. (2020). Exquisitely preserved fossil snakes of messel: Insight into the evolution, biogeography, habitat preferences and sensory ecology of early boas. *Diversity, 12*(3). https://doi.org/10.3390/d12030100

Scanferla, A., Smith, K. T., & Schaal, S. F. K. (2016). Revision of the cranial anatomy and phylogenetic relationships of the Eocene minute boas *Messelophis variatus* and *Messelophis ermannorum* (Serpentes, Booidea). *Zoological Journal of the Linnean Society, 176*(1), 182–206. https://doi.org/10.1111/zoj.12300

Scanferla, A., Zaher, H., Novas, F. E., de Muizon, C., & Céspedes, R. (2013). A new snake skull from the paleocene of bolivia sheds light on the evolution of macrostomatans. *PLoS ONE, 8*(3), 1–9. https://doi.org/10.1371/journal.pone.0057583

Scanlon, J. D. (1996). Studies in the palaeontology and systematics of australian snakes. [Doctoral dissertation, The University of New South Wales Sydney]. UNSW Sydney Library. https://www.unsworks.unsw.edu.au/primo-explore/fulldisplay/unsworks_70571/UNSWORKS

Scanlon, J. D. (1997). *Nanowana* gen. nov., small madtsoiid snakes from the miocene of Riversleigh: Sympatric species with divergently specialised dentition. *Memoirs of the Queensland Museum, 41*(2), 393–412.

Scanlon, J. D. (2003). The basicranial morphology of madtsoiid snakes (Squamata, Ophidia) and the earliest Alethinophidia (Serpentes). *Journal of Vertebrate Paleontology, 23*(4), 971–976. https://doi.org/10.1671/24

Scanlon, J. D. (2005). Cranial morphology of the Plio-Pleistocene giant madtsoiid snake *Wonambi naracoortensis*. *Acta Palaeontologica Polonica, 50*(1), 139–180.

Scanlon, J. D. (2006). Skull of the large non-macrostomatan snake *Yurlunggur* from the Australian Oligo-Miocene. *Nature, 439*(7078), 839–842. https://doi.org/10.1038/nature04137

Scanlon, J. D., & Lee, M. S. Y. (2000). The Pleistocene serpent *Wonambi* and the early evolution of snakes. *Nature, 403*(6768), 416–420. https://doi.org/10.1038/35000188

Scanlon, J. D., & Lee, M. S. Y. (2002). Varanoid-like dentition in primitive snakes (Madtsoiidae). *Journal of Herpetology, 36*(1), 100. https://doi.org/10.2307/1565810

Scanlon, J. D., Lee, M. S. Y., Caldwell, M. W., & Shine, R. (1999). The palaeoecology of the primitive snake *Pachyrhachis*. *Historical Biology, 13*(2–3), 127–152. https://doi.org/10.1080/08912969909386578

Schaal, S., & Baszio S. (2004). *Messelophis variatus* n. gen. nov. sp. from the Eocene of Messel: a tropidopheine snake with affinities to Erycinae (Boidae). *Courier Forschungsinstitut Senckenberg, 252,* 47–66.

Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Segall, M., Cornette, R., Fabre, A. C., Godoy-Diana, R., & Herrel, A. (2016). Does aquatic foraging impact head shape evolution in snakes? *Proceedings of the Royal Society B: Biological Sciences, 283*(1837). https://doi.org/10.1098/rspb.2016.1645

Senn, D. G., & Northcutt, R. G. (1973). The forebrain and midbrain of some squamates and their bearing on the origin of snakes. *Journal of Morphology, 140*(2), 135–151. https://doi.org/10.1002/jmor.1051400202

Sherratt, E., Coutts, F. J., Rasmussen, A. R., & Sanders, K. L. (2019a). Vertebral evolution and ontogenetic allometry: The developmental basis of extreme body shape divergence in microcephalic sea snakes. *Evolution and Development, 21*(3), 135–144. https://doi.org/10.1111/ede.12284

Sherratt, E., Sanders, K. L., Watson, A., Hutchinson, M. N., Lee, M. S. Y., & Palci, A. (2019b). Heterochronic shifts mediate ecomorphological convergence in skull shape of microcephalic sea snakes. *Integrative and Comparative Biology, 59*(3), 616–624. https://doi.org/10.1093/icb/icz033

Sheverdyukova, H. V. (2017). Development of the osteocranium in *Natrix natrix* (Serpentes, Colubridae) embryogenesis I: development of cranial base and cranial vault. *Zoomorphology, 136*(1), 131–143. https://doi.org/10.1007/s00435-016-0333-8

Sheverdyukova, H. V. (2019). Development of the osteocranium in *Natrix natrix* (Serpentes, Colubridae) embryogenesis II: Development of the jaws, palatal complex and associated bones. *Acta Zoologica, 100*(3), 282–291. https://doi.org/10.1111/azo.12253

Shorey, P. (1969). *Plato in Twelve Volumes (Vols. 5 & 6)*. Harvard University Press.

Shubin, N. (2009). *Your Inner Fish: A Journey into the 3.5-Billion-Year History of the Human Body*. Vintage.

Sidlauskas, B. (2008). Continuous and arrested morphological diversification in sister clades of characiform fishes: A phylomorphospace approach. *Evolution, 62*(12), 3135–3156. https://doi.org/10.1111/j.1558-5646.2008.00519.x

Silva, F. M. da, Oliveira, L. S. de, Nascimento, L. R. de S., Machado, F. A., & Prudente, A. L. da C. (2017). Sexual dimorphism and ontogenetic changes of Amazonian pit vipers (*Bothrops atrox*). *Zoologischer Anzeiger*, *271*, 15–24. https://doi.org/10.1016/j.jcz.2017.11.001

Silva, F. M., Prudente, A. L. da C., Machado, F. A., Santos, M. M., Zaher, H., & Hingst-Zaher, E. (2018). Aquatic adaptations in a Neotropical coral snake: A study of morphological convergence. *Journal of Zoological Systematics and Evolutionary Research, 56*(3), 382–394. https://doi.org/10.1111/jzs.12202

Simões, B. F., Sampaio, F. L., Douglas, R. H., Kodandaramaiah, U., Casewell, N. R., Harrison, R. A., Hart, N. S., Partridge, J. C., Hunt, D. M., & Gower, D. J. (2016a). Visual pigments, ocular filters and the evolution of snake vision. *Molecular Biology and Evolution, 33*(10), 2483–2495. https://doi.org/10.1093/molbev/msw148

Simões, B. F., Sampaio, F. L., Jared, C., Antoniazzi, M. M., Loew, E. R., Bowmaker, J. K., Rodriguez, A., Hart, N. S., Hunt, D. M., Partridge, J. C., & Gower, D. J. (2015a). Visual

system evolution and the nature of the ancestral snake. *Journal of Evolutionary Biology, 28*(7), 1309–1320. https://doi.org/10.1111/jeb.12663

Simões, T. R., Caldwell, M. W., Palci, A., & Nydam, R. L. (2017). Giant taxon-character matrices: Quality of character constructions remains critical regardless of size. *Cladistics, 33*(2), 198–219. https://doi.org/10.1111/cla.12163

Simões, T. R., Caldwell, M. W., Tałanda, M., Bernardi, M., Palci, A., Vernygora, O., Bernardini, F., Mancini, L., & Nydam, R. L. (2018). The origin of squamates revealed by a Middle Triassic lizard from the Italian Alps. *Nature, 557*(7707), 706–709. https://doi.org/10.1038/s41586-018-0093-3

Simões, T. R., Funston, G. F., Vafaeian, B., Nydam, R. L., Doschak, M. R., & Caldwell, M. W. (2016b). Reacquisition of the lower temporal bar in sexually dimorphic fossil lizards provides a rare case of convergent evolution. *Scientific Reports, 6*, 1–12. https://doi.org/10.1038/srep24087

Simões, T. R., Vernygora, O., Caldwell, M. W., & Pierce, S. E. (2020). Megaevolutionary dynamics and the timing of evolutionary innovation in reptiles. *Nature Communications, 11*(1), 1–14. https://doi.org/10.1038/s41467-020-17190-9

Simões, T. R., Wilner, E., Caldwell, M. W., Weinschütz, L. C., & Kellner, A. W. A. (2015b). A stem acrodontan lizard in the Cretaceous of Brazil revises early lizard evolution in Gondwana. *Nature Communications, 6*, 1–8. https://doi.org/10.1038/ncomms9149

Simpson, G. G. (1944). *Tempo and Mode in Evolution*. Columbia University Press.

Simpson, G. G. (1953). *The Major Features Of Evolution*. Columbia University Press.

Sites, J. W. (2011). Phylogenetic insights on evolutionary novelties in lizards and snakes: sex, birth, bodies, niches, and venom. *Annual Review of Ecology, Evolution, and Systematics, 42*, 222-244. https://doi.org/10.1146/annurev-ecolsys-102710-145051

Skawiński, T., & Borczyk, B. (2017). Evolution of developmental sequences in lepidosaurs. *PeerJ, (4)*. https://doi.org/10.7717/peerj.3262

Smith-Woodward, A. (1901). On some Extinct Reptiles from Patagonia, of the Genera *Miolania*, *Dinilysia*, and *Genyodecles*. *Proceedings of the Zoological Society of London, 1*, 169–184.

Smith, K. T. (2013). New constraints on the evolution of the snake clades Ungaliophiinae, Loxocemidae and Colubridae (Serpentes), with comments on the fossil history of erycine boids in North America. *Zoologischer Anzeiger - A Journal of Comparative Zoology, 252*(2), 157-182

Smith, K. T., & Scanferla, A. (2016). Fossil snake preserving three trophic levels and evidence for an ontogenetic dietary shift. *Palaeobiodiversity and Palaeoenvironments, 96*(4), 589–599. https://doi.org/10.1007/s12549-016-0244-1

Souto, N. M., Murta-Fonseca, R. A., Machado, A. S., Lopes, R. T., & Fernandes, D. S. (2019). Snakes as a model for measuring skull preparation errors in geometric morphometrics. *Journal of Zoology, 309*(1), 12–21. https://doi.org/10.1111/jzo.12678

Stalling, D., Westerhoff, M., & Hege, H. C. (2005). Amira: A highly interactive system for visual data analysis. *Visualization Handbook*, 749–767. https://doi.org/10.1016/B978-012387582-2/50040-X

Stayton, C. T. (2005). Morphological evolution of the lizard skull: A geometric morphometrics survey. *Journal of Morphology, 263*(1), 47–59. https://doi.org/10.1002/jmor.10288

Stayton, C. T. (2006). Testing hypotheses of convergence with multivariate data: Morphological and functional convergence among herbivorous lizards. *Evolution, 60*(4), 824. https://doi.org/10.1554/04-575.1

Stayton, C. T. (2008). Is convergence surprising? An examination of the frequency of convergence in simulated datasets. *Journal of Theoretical Biology, 252*(1), 1–14. https://doi.org/10.1016/j.jtbi.2008.01.008

Stayton, C. T. (2015). The definition, recognition, and interpretation of convergent

evolution, and two new measures for quantifying and assessing the significance of convergence. *Evolution, 69*(8), 2140–2153.

Sterelny, K. (2003). *Dawkins vs. Gould: Survival of the Fittest*. Icon Books

StreetWise Media. 2019. A Leg Up For Snake Evolution. Available at https://www.streetwisemedia.com.au/fossil-shakes-a-leg-for-snake-evolution/

Streicher, J. W., & Wiens, J. J. (2017). Phylogenomic analyses of more than 4000 nuclear loci resolve the origin of snakes among lizard families. *Biology Letters, 13*, 20170393. https://doi.org/10.1098/rsbl.2017.0393

Strong, C. R. C., Palci, A., & Caldwell, M. W. (2020). Insights into skull evolution in fossorial snakes, as revealed by the cranial morphology of *Atractaspis irregularis* (Serpentes: Colubroidea). *Journal of Anatomy*, 1–27. https://doi.org/10.1111/joa.13295

Strong, C. R. C., Simões, T. R., Caldwell, M. W., & Doschak, M. R. (2019). Cranial ontogeny of *Thamnophis radix* (Serpentes: Colubroidea) with a re-evaluation of current paradigms of snake skull evolution. *Royal Society Open Science, 6*(8). https://doi.org/10.1098/rsos.182228

Stroud, J. T., & Losos, J. B. (2016). Ecological opportunity and adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics, 47*, 507–532. https://doi.org/10.1146/annurev-ecolsys-121415-032254

Szyndlar, Z., & Böhme, W. (1996). Redescription of *Tropidonotus atavus* von Meyer, 1855 from the upper Oligocene of Rott (Germany) and its allocation to *Rottophis* gen. nov. (Serpentes, Boidae). *Palaeontogra–phica Abteilungen A, 240*, 145–161.

Tamagnini, D., Stephenson, J., Brown, R. P., & Meloro, C. (2018). Geometric morphometric analyses of sexual dimorphism and allometry in two sympatric snakes: *Natrix helvetica* (Natricidae) and *Vipera berus* (Viperidae). *Zoology, 129*, 25–34. https://doi.org/10.1016/j.zool.2018.05.008

Tatarinov, L. P. (1963). First discovery of ancient sea snakes in the USSR. *Paleontol. Zh., 2*, 109-115 [in Russian].

Tatarinov, L. P. (1988). Structure of the skull of the sea snake "*Archaeophis*" *turkmenicus* from the lower Eocene of Turkmenia. *Paleontol. Zh., 1*, 75-82 [in Russian].

Tayhan, Y., Yakin, B. Y., & Tok, C. V. (2016). Shape variability of the head of *Ophisops elegans* Ménétriés, 1832 (Reptilia: Lacertidae) from Konya, Turkey. *Italian Journal of Zoology, 83*(2), 208–212. https://doi.org/10.1080/11250003.2016.1153163

Taylor, G. W., Santos, J. C., Perrault, B. J., Morando, M., Vásquez Almazán, C. R., & Sites, J. W. (2017). Sexual dimorphism, phenotypic integration, and the evolution of head structure in casque-headed lizards. *Ecology and Evolution, 7*(21), 8989–8998. https://doi.org/10.1002/ece3.3356

Taylor, T. (1801). *The metaphysics of Aristotle: translated from the greek; with copious notes, in which the Phythagoric and Platonic dogmas respecting numbers and ideas unfolded antient sources (Classic reprint)*. London: printed for the author, by Davis, Wilks and Taylor. https://archive.org/details/metaphysicsofari00aris

Tchernov, E., Rieppel, O., Zaher, H., Polcyn, M. J., & Jacobs, L. L. (2000). A fossil snake with limbs. *Science, 287*(5460), 2010–2012. https://doi.org/10.1126/science.287.5460.2010

Thompson, D'A. W. (1910). *Historia Animalium (The History of Animals) (translation)*. Clarendon Press. Available at http://classics.mit.edu//Aristotle/history_anim.html

Thompson, D'A. W. (1917). *On Growth and Form (1st ed.)*. Cambridge University Press.

Triviño, L. N., Albino, A. M., Dozo, M. T., & Williams, J. D. (2018). First natural endocranial cast of a fossil snake (Cretaceous of Patagonia, Argentina). *Anatomical Record, 301*(1), 9–20. https://doi.org/10.1002/ar.23686

Tschopp, P., Sherratt, E., Sanger, T. J., Groner, A. C., Aspiras, A. C., Hu, J. K., Pourquié, O., Gros, J., & Tabin, C. J. (2014). A relative shift in cloacal location repositions

external genitalia in amniote evolution. *Nature, 516*(7531), 391–394. https://doi.org/10.1038/nature13819

Tucker, A. S. (2017). Major evolutionary transitions and innovations: The tympanic middle ear. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1713). https://doi.org/10.1098/rstb.2015.0483

Uetz, P. & Hošek, J. (2020). The reptile database. Available at http://www.reptile-database.org

Underwood, G. (1967). *A Contribution to the Classification of Snakes*. London: British Museum Natural History Publications.

Underwood, G. (1970). The eye. In C. Gans, & T.S. Parsons (Eds.), *Biology of the Reptilia* (Volume 2, Morphology B, pp 1-97). Academic Press, New York.

Urošević, A., Ljubisavljević, K., & Ivanović, A. (2013). Patterns of cranial ontogeny in lacertid lizards: Morphological and allometric disparity. *Journal of Evolutionary Biology, 26*(2), 399–415. https://doi.org/10.1111/jeb.12059

Urošević, A., Ljubisavljević, K., & Ivanović, A. (2014). Variation in skull size and shape of the Common wall lizard (*Podarcis muralis*): allometric and non-allometric shape changes. *Contributions to Zoology*, *83*(1), 67-S1. https://doi.org/10.1163/18759866-08301003

Urošević, A., Ljubisavljević, K., & Ivanović, A. (2019). Multilevel assessment of the Lacertid lizard cranial modularity. *Journal of Zoological Systematics and Evolutionary Research, 57*(1), 145–158. https://doi.org/10.1111/jzs.12245

Van Valen, L. (1974). A natural model for the origin of some higher taxa. *Journal of Herpetology, 8*(2), 109-121

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer.

Verma, S. K., & Puneka, R. M. (2019). Chapter 74: Observing nature—what designers can learn from biologists. In A. Chakrabarti (Ed.), *Research into Design for a Connected World* (pp. 887-899). Verlag: Springer Singapore. https://doi.org/10.1007/978-981-13-5977-4_74

Vidal, M. A., Veloso, A., & Méndez, M. A. (2006). Insular morphological divergence in the lizard *Liolaemus pictus* (Liolaemidae). *Amphibia Reptilia, 27*(1), 103–111. https://doi.org/10.1163/156853806776052146

Vidal, N., & Hedges, S. B. (2004). Molecular evidence for a terrestrial origin of snakes. *Proceedings of the Royal Society B: Biological Sciences, 271*(4). https://doi.org/10.1098/rsbl.2003.0151

Vidal, N., & Hedges, S. B. (2005). The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein-coding genes. *Comptes Rendus - Biologies, 328*(10–11), 1000–1008. https://doi.org/10.1016/j.crvi.2005.10.001

Vidal, N., & Hedges, S. B. (2009). The molecular evolutionary tree of lizards, snakes, and amphisbaenians. *Comptes Rendus - Biologies, 332*(2–3), 129–139. https://doi.org/10.1016/j.crvi.2008.07.010

von Baer, K. E. (1828). *Uber entwickelungsgeschichte der thiere: beobachtung und reflexion*. Königsberg, Bei den Gebrüdern Borntträger.

Vonk, F. J., Admiraal, J. F., Jackson, K., Reshef, R., De Bakker, M. A. G., Vanderschoot, K., Van Den Berge, I., Van Atten, M., Burgerhout, E., Beck, A., Mirtschin, P. J., Kochva, E., Witte, F., Fry, B. G., Woods, A. E., & Richardson, M. K. (2008). Evolutionary origin and development of snake fangs. *Nature, 454*(7204), 630–633. https://doi.org/10.1038/nature07178

Voronov, A. S., Shibalev, D. V., & Kupriyanova, N. S. (2011). Evolutionary relationships between reptiles inferred from the comparison of their ITS2 sequences. *Russian Journal of Genetics, 47*(7), 864–873. https://doi.org/10.1134/S1022795411060160

Vullo, R. (2019). A new species of *Lapparentophis* from the mid-Cretaceous Kem Kem beds, Morocco, with remarks on the distribution of lapparentophiid snakes. *Comptes*

Rendus - Palevol, 18(7), 765–770. https://doi.org/10.1016/j.crpv.2019.08.004

Wagner, G. P., Pavlicev, M., & Cheverud, J. M. (2007). The road to modularity. *Nature Reviews Genetics, 8*(12), 921–931. https://doi.org/10.1038/nrg2267

Wake, D.B., Wake, M. H., & Specht, C. D. (2011). Homoplasy: From detecting pattern to determining process and mechanism of evolution. *Science, 331*, 1032-1035.DOI: 10.1126/science.1188545

Wallach, V. (1984). A New Name for *Ophiomorphus colberti* Haas, 1980. *Journal of Herpetology., 18*(3), 329.

Wallach, V., Williams, K. L., & Boundy, J. (2014). *Snakes of the World: A Catalogue of Living and Extinct Species* (1st ed.). CRC Press.

Walls, G. (1942). *The Vertebrate Eye and its Adaptive Radiation*. Hafner, New York.

Walls, G. L. (1940). Ophthalmological implications for the early history of the snakes. *Copeia, 1940*(1), 1. https://doi.org/10.2307/1439015

Watanabe, A., Fabre, A. C., Felice, R. N., Maisano, J. A., Müller, J., Herrel, A., & Goswami, A. (2019). Erratum: Ecomorphological diversification in squamates from conserved pattern of cranial integration (Proceedings of the National Academy of Sciences of the United States of America (2019) 116 14688–14697) DOI: 10.1073/pnas.1820967116). *Proceedings of the National Academy of Sciences of the United States of America, 116*(34), 17129. https://doi.org/10.1073/pnas.1912325116

Webster, A. J., & Purvis, A. (2002). Testing the accuracy of methods for reconstructing ancestral states of continuous characters. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1487), 143–149. https://doi.org/10.1098/rspb.2001.1873

Werneburg, I., & Sánchez-Villagra, M. R. (2015). Skeletal heterochrony is associated with the anatomical specializations of snakes among squamate reptiles. *Evolution, 69*(1), 254–263. https://doi.org/10.1111/evo.12559

Werneburg, I., Polachowski, K. M., & Hutchinson, M. N. (2015). Bony skull development in the Argus monitor (Squamata, Varanidae, *Varanus panoptes*) with comments on developmental timing and adult anatomy. *Zoology, 118*(4), 255–280. https://doi.org/10.1016/j.zool.2015.02.004

Wever, E. G. (1968). The ear of the chameleon: *Chamaeleo senegalensis*. *Journal of Experimental Zoology, 168*, 423–436.

Wiens, J. J., & Slingluff, J. L. (2001). How lizards turn into snakes: A phylogenetic analysis of body-form evolution in anguid lizards. *Evolution, 55*(11), 2303–2318. https://doi.org/10.1111/j.0014-3820.2001.tb00744.x

Wiens, J. J., Brandley, M. C., & Reeder, T. W. (2006). Why does a trait evolve multiple times within a clade? repeated evolution of snakelike body form in squamate reptiles. *Evolution, 60*(1), 123. https://doi.org/10.1554/05-328.1

Wiens, J. J., Hutter, C. R., Mulcahy, D. G., Noonan, B. P., Townsend, T. M., Sites, J. W., & Reeder, T. W. (2012). Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. *Biology Letters, 8*(6), 1043–1046. https://doi.org/10.1098/rsbl.2012.0703

Wiens, J. J., Kuczynski, C. A., Townsend, T., Reeder, T. W., Mulcahy, D. G., & Sites, J. W. (2010). Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: Molecular data change the placement of fossil taxa. *Systematic Biology, 59*(6), 674–688. https://doi.org/10.1093/sysbio/syq048

Wilson, J. A., Mohabey, D. M., Peters, S. E., & Head, J. J. (2010). Predation upon hatchling dinosaurs by a new snake from the Late Cretaceous of India. *PLoS Biology, 8*(3), e1000322. https://doi.org/10.1371/journal.pbio.1000322

Woltering, J. M. (2012). From lizard to snake: behind the evolution of an extreme body plan. *Current Genomics, 13*(4), 289–299. https://doi.org/10.2174/138920212800793302

Xing, L., Caldwell, M. W., Chen, R., Nydam, R. L., Palci, A., Simões, T. R., McKellar, R. C.,

Lee, M. S. Y., Liu, Y., Shi, H., Wang, K., & Bai, M. (2018). A mid-Cretaceous embryonic-to-neonate snake in amber from Myanmar. *Science Advances, 4*(7), 1–9. https://doi.org/10.1126/sciadv.aat5042

Yi, H., & Norell, M. A. (2015). Evolutionary Biology: The burrowing origin of modern snakes. *Science Advances, 1*(10). https://doi.org/10.1126/sciadv.1500743

Young, N. M., Hu, D., Lainoff, A. J., Smith, F. J., Diaz, R., Tucker, A. S., Trainor, P. A., Schneider, R. A., Hallgrímsson, B., & Marcucio, R. S. (2014). Embryonic bauplans and the developmental origins of facial diversity and constraint. *Development (Cambridge), 141*(5), 1059–1063. https://doi.org/10.1242/dev.099994

Zaher, H. (1998). The phylogenetic position of *Pachyrhachis* within snakes (Squamata, Lepidosauria). *Journal of Vertebrate Paleontology, 18*(1), 1–3. https://doi.org/10.1080/02724634.1998.10011029

Zaher, H., & Rieppel, O. (1999). The phylogenetic relationships of *Pachyrhachis problematicus*, and the evolution of limblessness in snakes (Lepidosauria, Squamata). *Comptes Rendus de l'Academie de Sciences - Serie IIa: Sciences de La Terre et Des Planètes, 329*(11), 831–837.

Zaher, H., & Rieppel, O. (2000). A brief history of snakes. *Herpetological Review, 31*(2), 73–76.

Zaher, H., & Rieppel, O. (2002). On the phylogenetic relationships of the cretaceous snakes with legs, with special reference to *Pachyrhachis problematicus* (Squamata, Serpentes). *Journal of Vertebrate Paleontology, 22*(1), 104-109.

Zaher, H., & Scanferla, C. A. (2012). The skull of the Upper Cretaceous snake *Dinilysia patagonica* Smith-Woodward, 1901, and its phylogenetic position revisited. *Zoological Journal of the Linnean Society, 164*(1), 194–238. https://doi.org/10.1111/j.1096-3642.2011.00755.x

Zaher, H., Apesteguía, S., & Scanferla, C. A. (2009). The anatomy of the Upper Cretaceous snake *Najash rionegrina* Apesteguía & Zaher, 2006, and the evolution of limblessness in snakes. *Zoological Journal of the Linnean Society, 156*(4), 801–826. https://doi.org/10.1111/j.1096-3642.2009.00511.x

Zaher, H., Murphy, R. W., Arredondo, J. C., Graboski, R., Machado-Filho, P. R., Mahlow, K., Montingelli, G. G., Quadros, A. B., Orlov, N. L., Wilkinson, M., Zhang, Y. P., & Grazziotin, F. G. (2019). Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). *PLoS ONE, 14*(5). https://doi.org/10.1371/journal.pone.0216148

Zahradnicek, O., Buchtova, M., Dosedelova, H., & Tucker, A. S. (2014). The development of complex tooth shape in reptiles. *Frontiers in Physiology, 5*, 74. https://doi.org/10.3389/fphys.2014.00074

Zelditch, M. L. (Ed.). (2001). *Beyond Heterochrony: The Evolution of Development*. John Wiley & Sons, Inc.

Zelditch, M. L., Swiderski, D. L., H. Sheets, D., & Fink, W. L. (2004). *Geometric Morphometrics For Biologists: A Primer*. Academic Press.

Zheng, Y., & Wiens, J. J. (2016). Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Molecular Phylogenetics and Evolution, 94*, 537–547. https://doi.org/10.1016/j.ympev.2015.10.009

Zimm, R. (2019). On the development of the turtle scute pattern and the origins of its variation. [Doctoral dissertation, University of Helsinki]. Helda database. https://helda.helsinki.fi/handle/10138/299142

Zuffi, M. A. L., Sacchi, R., Pupin, F., & Cencetti, T. (2011). Sexual size and shape dimorphism in the Moorish gecko (*Tarentola mauritanica*, Gekkota, Phyllodactylidae). *North-Western Journal of Zoology*, *7*(2), 189–197.

Phyllodactylidae). *North-Western Journal of Zoology*, *7*(2), 189–197.